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CERCARIAL PENETRATION STUDIES: STEPS TOWARD CHEMOPROPHYLAXIS IN SCHISTOSOMIASIS

ANNUAL AND FINAL REPORT

B. SALAFSKY AND A.C. FUSCO

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U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report summarizes the research we have conducted during the three year scope of DAMD Contract #17-85-C-5180. The focus of this research centered on elucidating the biochemical mechanisms involved in cercarial (Schistosoma mansoni) skin penetration and the evaluation of eicosanoid inhibitors as possible prophylactic agents. Nineteen areas of research were pursued during these three years: 1) The effect of low linoleate concentration on cercarical penetration and transformation. 2) The role of pH in cercarial eicosanoid production. 3) The effect of ibuprofen and esculetin on cercarial penetration, transformation and eicosanoid production. 4) Validation of the [^{75}Se] labelling technique for tracking cercarial penetration. 5) The e-fect of esculetin on cercarial penetration and transformation in vivo. 6) The biochemical mechanisms involved in cercarial transformation. 7) The role of skin eicosanoid production in cercarial penetration. 8) Drug screening of various eicosanoid inhibitors. 9) Experiments on the use of an artificial skin membrane to investigate cercarial penetration in vitro. 10) The use of ketoconazole as a prophylactic agent.

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19. 11) The effect of praziquantel on cercarial penetration, transformation and eicosanoid production in vitro and in vivo. 12) The effects of Cremophor EL on cercariae in vitro. 13) Cercarial stimulation & inhibition by Castor Oil & ricinoleic acid. 14) Inhibition of cercarial eicosanoid production via Cremophor EL, Castor Oil, ricinoleate. 15) The effect of Cremophor EL, Castor Oil, & ricinoleic acid as prophylactic agents. 16) Free fatty acid analysis of skin surface after treatment with Cremophor EL, Castor Oil & ricinoleic acid. 17) The inhibition of cercarial penetration by skin lipid changes. 18) The in vivo Effect of 2 ricinoleate-like compounds. 19) Eicosanoid production in adult schistosomes. Of the compounds investigated for prophylactic activity in vivo, Castor Oil proved superior; however, its clinical applications are doubtful. This compound does, however give us a structure-activity basis from which to search for more promising compounds. In addition, it clearly indicates that chemoprophylaxis to schistosomiasis is possible.

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SUMMARY

This report summarizes the research we have conducted during the entire scope of contract #DAMD17-85-C-5180 (15 August 1985 to 14 August 1988). The scope of this research centered on elucidating the biochemical mechanisms involved in cercarial (Schistosoma mansoni) skin penetration and the evaluation of eicosanoid inhibitors as possible prophylactic drugs. During the past three years, 19 areas of research were pursued:

1. The Effect of Low Linoleate Concentration on Cercarial Penetration and Transformation. We have concluded that while cercarial penetration and transformation rates increased over time when exposed to very low levels of linoleate, this increase cannot be correlated to either eicosanoid production or phospholipid levels. Since cercarial eicosanoid levels do correlate with increasing concentrations of linoleate, we have concluded that using low linoleate concentrations to help elucidate the biochemistry of cercarial skin penetration was not a fruitful approach.

2. Role of pH in Cercarial Eicosanoid Production. We have found that pH directly affects cercarial eicosanoid production when agar is utilized as a penetration substrate. Cercarial eicosanoid production is increased at slightly acidic pH when compared to slightly alkaline pH.

3. The Effect of Ibuprofen and Esculetin on Cercarial Penetration, Transformation and Eicosanoid Production (in vitro). Ibuprofen has no effect on cercarial penetration or transformation rates at concentrations as high as 10mM when it is incorporated into a gelatin:agar matrix. Esculetin (1mM) was effective at inhibiting cercarial penetration, transformation and eicosanoid production; however, its ability to inhibit either process was pH dependent.

4. Validation of the [^{75}Se] Labelling Technique for Tracking Cercarial Penetration and Transformation in vivo. We validated the technique of using [^{75}Se] labelled cercariae for the in vivo measurement of cercarial penetration and migration in ICR mice.

5. The Effect of Esculetin on Cercarial Penetration and Transformation in vivo. Esculetin concentrations of 100mg/kg or 200mg/kg failed to protect mice when challenged with cercariae via the tail route; most probably due to its short serum half-life. Thus, esculetin was ruled out as a possible prophylactic agent.

6. The Biochemical Mechanisms Involved in Cercarial Transformation. An analysis of various cercarial transformation methods in relation to ultrastructure, eicosanoid production, RNA, DNA, and protein synthesis, and loss of water tolerance was undertaken. We found that while no real ultrastructural differences between all transformation methods existed with respect to schistosomulae, there were considerable biochemical differences between transfor-

mation methods. In general, mechanically transformed schistosomulae appeared to be biochemically damaged. We suggest that chemical transformation methods be used for all experiments dealing with early schistosomulae.

7. The Role of Skin Eicosanoid Production in Cercarial Penetration. We correlated skin HETE production to cercarial penetration in eight strains of mice and 1 strain of rat. Thus we defined a biochemical basis for cercarial penetration involving host skin eicosanoid production. These experiments lend further support to our "eicosanoid hypothesis".

8. Drug Screening of Various Eicosanoid Inhibitors. We screened 42 eicosanoid inhibitors for their effect, in vitro, on cercarial stimulation by linoleate. Based on these efforts we decided to investigate the following drugs for in vitro activity in inhibiting cercarial penetration: praziquantel, ketoconazole and sulfasalazine. Various derivatives of these drugs were also evaluated in in vitro screens.

9. Experiments on the Use of an Artificial Skin Membrane to Investigate Cercarial Penetration and Transformation Mechanisms in vitro. Cercariae were not able to penetrate an artificial skin membrane composed of keratin:chitin or keratin:collagen; thus, we stopped pursuing the development of the "Widra" membrane. However, because we believed that the development of an artificial penetration substrate was a key element in in vitro drug screening, we investigated other membrane formulations including overlaying membranes with human keratinocytes. Unfortunately, no membrane preparation was found to be satisfactory for in vitro drug screening.

10. The Use of Ketoconazole as a Prophylactic Agent. We tested ketoconazole as an oral chemoprophylactic agent given its effectiveness in in vitro drug screening, its reported effects as a strong 5-lipoxygenase inhibitor, and our correlation of cercarial skin penetration with lipoxygenase products. A single dose of 200mg/kg ketoconazole was not effective in inhibiting cercarial penetration at either 2, 4, 8, or 16 hrs after drug administration. Since detectable levels of ketoconazole can be found at the surface of the skin within 8 hrs following a single dose, we did not pursue further investigations on this drug.

11. The Effect of PZQ on Cercarial Penetration, Transformation and Eicosanoid Production in vitro and in vivo: Drug trials with PZQ indicated that this drug held promise as a prophylactic agent; however, its effectiveness was clearly vehicle-dependent. The effectiveness of Cremophor EL (a lipid vehicle) without PZQ, suggested that a combination of dietary and chemoprophylactic methods may be worth exploring. This lead us to concentration on the prophylactic effect of Cremophor El and its derivatives during the last year of this contract.

12. The Effects of Cremophor EL on Cercariae in vitro: Cremophor El (ethoyxlated Castor Oil) inhibited cercarial response to linoleate; however, this response was NOT dose dependent. In addition, cercariae were not stimulated by Cremophor El alone, nor did it have a strong ability to 'mask' linoleate when applied directly over it.

13. Cercarial Stimulation & Inhibition by Castor Oil & Ricinoleic Acid. We investigated whether Castor Oil and Ricinoleate could either stimulate cercarial transformation or mask cercarial stimulation by linoleate, in order to discern the role these lipids might have in preventing cercarial penetration. Castor Oil was not stimulatory (i.e.able to induce transformation events leading to loss of water tolerance) to cercariae, nor was it able to completely mask cercarial stimulation by linoleate (~24% reduction). Ricinoleate, however, was as stimulatory as linoleate after 60 min exposure. Thus, we decided to further investigate these compounds.

14. Inhibition of Cercarial Eicosanoid Production via Cremophor El, Castor Oil, & Ricinoleate. We evaluated the effect of Cremophor El, Castor Oil, 80% pure ricinoleate and 99% pure ricinoleate on cercarial eicosanoid production. Cremophor El gave 100% inhibition of cercarial eicosanoid production, while Castor Oil and ricinoleate showed much less inhibition. However, both Castor Oil and ricinoleate are less soluble in aqueous solutions than is Cremophor El (a compound formulated to have higher water solubility). Thus, the results obtained with Castor Oil and ricinoleate can only be regarded as TENTATIVE until these experiments can be repeated using sonicated and emulsified ELAC-oil solutions.

15. The Effect of Cremophor El, Castor Oil, & Ricinoleic Acid as Prophylactic Agents. We evaluated the chemoprophylactic effects of Castor Oil and ricinoleic acid as compared to Cremophor El since the in vitro effects of Castor Oil and ricinoleate and the in vivo effects of Cremophor El were so promising. Castor Oil, at a concentration of 9.8g/kg, proved to be the most effective of these compounds. This lipid compound was extremely effective at inhibiting cercarial penetration

16. Free Fatty Acid Analysis of Skin Surface After Treatment with Cremophor El, Castor Oil & Ricinoleic Acid. We examined the surface lipid composition of mouse tail skin in order to determine if treatment with Castor Oil, Cremophor El or ricinoleate caused skin lipid changes. Both ricinoleate and Castor Oil caused the surface lipid composition of mouse tail skin to be altered. Thus, increasing dietary intake of certain lipid can cause skin lipid changes which could possibly inhibit cercarial penetration (see #17).

17. Inhibition of Cercarial Penetration by Skin Lipid Changes. We topically treated the tail skin of mice with oleate, Cremophor El, or linoleate in order to determine if skin lipid changes can inhibit or promote cercarial penetration. The data showed that lipid changes on the surface of the tail can cause dramatic changes in cercarial penetration indicating that skin surface lipid perturbations can result in inhibition of cercarial penetration.

18. General Comments on the Chemoprophylactic Effect of Castor Oil. Near the end of this contract our COTR supplied us with a structure report detailing well over 100 compounds having a similar structure to ricinoleate. We were able to obtain two compounds in sufficient quantities and in enough time to evaluate them in vivo before this contract expired. Unfortunately neither AJ00983 nor AG12723 were able to inhibit cercarial penetration. Structure activity relations of these compounds, in comparison to ricinoleate, have provided valuable clues to the design of future prophylactic agents.

19. Eicosanoid Production in Adult Schistosomes: Adult schistosomes secrete immunosuppressive eicosanoids of both the cyclo-oxygenase and lipoxygenase pathways. PGE has been detected by both HPLC and RIA. The HPLC data also show that the immunosuppressant 15-HETE is present in amounts exceeding that of the immunostimulant 5-HETE. In addition, other eicosanoids having potentially positive or negative effects on the immune system are also present. While we do not believe, at this time, that eicosanoid production is the major mechanism responsible for keeping the adults and larval stages of the parasite "hidden" from the host immune system, we do believe that the evidence presented here suggest a supportive role for these products. These data further strengthen our hypothesis that eicosanoids play a key role in cercarial invasion and maturation in the host.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

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STATEMENT OF PROBLEM

Schistosomiasis is a parasitic, debilitating infectious disease endemic in tropical and semi-tropical areas of the world. Many of these areas are of potential military interest (i.e. South America, North, Central, West Africa and the Near East) due to unstable governments, vital raw materials, and incursion by powers unfriendly to the United States. There currently does not exist an effective prophylaxis, therapeutically or at the vaccine level for schistosomiasis. The combat soldier and possible support personnel serving in these regions are therefore at risk of infection. The studies in progress for this contract afford an opportunity to more fully understand the biochemical mechanisms involved in the cercarial (see glossary) skin penetration and transformation, the first step in infection. With such a database in place, we believe it will be possible to rationally develop a novel approach to chemoprophylaxis in schistosomiasis. This contract centers on cercarial stimulation by essential fatty acids and resultant eicosanoid production as a possible specific target of chemoprophylactic attack.

THE BACKGROUND

We have demonstrated that cercarial eicosanoids (lipoxygenase and cyclo-oxygenase products) produced as the result of stimulation by skin surface essential fatty acids (EFA) are important mediators for successful cercarial penetration and transformation. These findings have been supported by: 1) the stimulatory nature of EFA and their role in eicosanoid production, 2) the reduction in penetration response and transformation when cercariae are treated with eicosanoid inhibitors, 3) the wide variety of eicosanoids produced when cercariae are stimulated by EFA, 4) the ability of eicosanoid inhibitors to dramatically decrease cercarial eicosanoid production, 5) the dose-response nature of eicosanoid stimulation by EFA, 6) the correlation of cercarial penetration with lipoxygenase products (i.e. LTs and HETEs) and 7) the identification of LTB_4 or metabolites as correlates of the penetration process. Given these data we have formulated an "eicosanoid hypothesis" that states: (1) Cercariae are stimulated to penetrate skin via skin surface essential fatty acids. (2) The fatty acids are incorporated by cercariae and serve to trigger cercarial eicosanoid production. (3) These eicosanoids either act directly within cercariae to play a vital role in the biochemical events associated with transformation and/or are secreted by cercariae into the host, resulting in immuno-modulatory influences that favor penetration.

APPROACH TO THE PROBLEM

Given the Statement of Problem and Background as detailed above, this contract sought to approach the problem of chemoprophylaxis in schistosomiasis via:

a) Conducting preliminary investigations on the in vivo prophylactic efficacy of various eicosanoid inhibitors.

b) Studying the role of skin EFAs, other skin lipid components and cercarial eicosanoid production in relation to the cercarial penetration and/or transformation responses and drug intervention.

c) Studying the mechanisms of action of ibuprofen, esculetin, and related eicosanoid inhibitors.

NARRATIVE SUMMARY (RESULTS & DISCUSSION) OF RESEARCH PERFORMED FROM 14 AUGUST 1985 - 14 AUGUST 1988.

Given the Approach to the Problem as defined above and in our contract, the following 19 areas of research were conducted from 14 August 1985 to 14 August 1988.

1. The Effect of Low Linoleate Concentration of Cercarial Penetration and Transformation.

Initially, we determined that cercariae exhibited a dose-response phenomena with respect to transformation, penetration and eicosanoid production when exposed to varying concentrations of linoleate for 1 hr at 37C. Low linoleate concentrations (i.e 0.003 and 0.03mM), resulted in minimal penetration, transformation, and eicosanoid synthesis rates. Increasing the linoleate concentration from 0.003 to 9mM resulted in an increase in penetration rates, LT levels, and HETE levels until 1mM linoleate is reached when transformation rates and PG production also begin to increase. At 9mM a toxic concentration of linoleate was reached. Since these experiments were conducted by exposing cercariae to linoleate for 1 hr, we wondered whether cercariae would respond to low linoleic acid levels (0.003 & 0.03mM) if the time of exposure was increased. Thus we measured cercarial penetration, transformation and eicosanoid production after 1, 2, and 4 hrs exposure to either 0.003 or 0.03mM linoleate. Our data showed that at 0.003mM linoleate, the percentage of cercariae penetrating an agar:gelatin matrix gradually increased from 0% at 1 hr to a high of 70% after 4 hrs at 37°C; however, transformation rates did not change. Control plates containing no linoleate did not show an increase in penetration rates. At 0.03mM linoleate, the percentage of cercariae penetrating an agar:gelatin matrix increased from 25 to 55% between 1 and 4 hrs, while transformation rates increased from 20 to 40% over the same time period. Thus it appears that cercariae can respond to low linoleate concentrations over time; however, the time periods involved suggest that this is not a physiological response (Cercariae can normally penetrate skin within 5 mins). This interpretation is also supported by HPLC analysis of cercarial eicosanoid and phospholipid production in the above experiments. Neither phospholipid nor eicosanoid levels were significantly correlated to cercarial penetration or transformation. In fact eicosanoid and phospholipid synthesis were minimal. However, all

the experiments reported above were conducted between pH 7.2 and 7.5 and we have since found eicosanoid production to be pH dependent, favoring a slightly acidic pH (see #2 below). Therefore, the possibility exists that different results may be obtained using a lower pH and/or RIA assay instead of HPLC as a measure of eicosanoid production (RIA is more sensitive). However, we felt that continuing this line of research would not enable us to examine the early biochemical steps involved in cercarial skin penetration, as we had hoped. Hence this line of research was discontinued.

2. Role of pH in Cercarial Eicosanoid Production.

We noted that the cercarial transformation process is pH sensitive. Cercarial penetration through an agar:gelatin matrix containing 3mM linoleate was 90-100% complete over a pH range of 5.5 to 8.0. However, transformation of cercariae occurred optimally between pH 6.2 and 7.3. Measurement of overall eicosanoid production showed that eicosanoid synthesis was greater at pH 6.55 than 7.2. These experiments demonstrate the importance of maintaining accurate pH levels in all experiments involving cercarial penetration and transformation.

3. The Effect of Ibuprofen and Esculetin on Cercarial Penetration, Transformation and Eicosanoid Production (in vitro).

We completed a series of experiments testing the effect of both ibuprofen and esculetin on cercarial penetration, transformation and eicosanoid production in vitro using our gelatin:agar plate methodology. Previously, these experiments were done in a liquid ELAC medium containing 3mM linoleate (37°C, 1 hr, 13000 cercariae) with varying concentrations of either ibuprofen or esculetin. We have found dramatic differences using the agar:gelatin substrate and otherwise identical conditions. For example, ibuprofen was not effective at inhibiting either penetration or transformation at concentrations as high as 10mM at a pH of 6.0 or 7.2 when incorporated into an agar:gelatin matrix; however, ibuprofen is effective at much lower concentrations when cercariae are incubated in a liquid medium (ELAC).

Esculetin affects cercariae differently depending on the pH of the agar:gelatin substrate. At pH 6.6 esculetin (1mM) inhibits penetration, but at more alkaline pH levels, it has no effect on penetration; rather, it preferentially inhibits transformation. Cercarial transformation rates fell below control levels at pH values less than 6.4 and greater than 6.7 but remained normal in the range of 6.4 to 6.7. However, cercarial penetration decreased in this pH 6.4 to 6.7 range, but otherwise remained at control levels. When the effect of esculetin on cercarial eicosanoid was measured at pH 6.55 and pH 7.2, we noted an overall decrease in eicosanoid synthesis. However, at pH 7.2, two eicosanoids with retention times of 38'38" (Lt region) and 1:02 (HETE region) were found in increased amounts when compared to controls. This finding

is particularly significant considering that cercariae exposed to esculetin at pH 7.2 have normal penetration while those exposed to esculetin at pH 6.55 have reduced penetration rates. Thus, we believe that we have identified two eicosanoids, one LT and one HETE, that are involved in the penetration process. In addition, we note that both cercarial transformation and eicosanoid production are pH dependent. A change of less than 1 pH unit can cause a shift in the species of eicosanoid produced as well as have dramatic effects on cercarial transformation. Further analysis revealed that LTB_4 and/or its metabolites are the leukotriene species involved in the penetration process.

4. Validation of the 75[Se] Labelling Technique for Tracking Cercarial Penetration and Transformation in vivo.

After obtaining a [^{75}Se] license we ran numerous studies evaluating the technique of using [^{75}Se] labelled cercariae for the measurement of cercarial penetration and migration. In our hands, using the ICR mouse strain, between 78.5 and 87.9% of labelled cercariae penetrate mouse tail skin. Of these ~77.6% are found in the lungs at 7 days, and 18.5% remained in tail skin (dark dots on autoradiography). These results compare favorably with previously published data; hence this technique has been validated in our laboratory.

5. The Effect of Esculetin on Cercarial Penetration and Transformation in vivo.

We completed evaluating a series of experiments in which the effect of various esculetin concentrations on cercarial penetration were studied in vivo. The experiments were setup according to the following goals: a) to develop an effective esculetin HPLC assay that could be used to determine esculetin levels in mouse skin & blood, b) to determine normal (untreated) cercarial penetration and migration rates in mice using the [^{75}Se] labeling technique, and c) to determine cercarial penetration and migration rates in mice using the [^{75}Se] labeling technique given an esculetin dose regime.

a) Development of an HPLC technique for measuring esculetin levels in skin and plasma. The method we developed for the extraction of esculetin from blood and skin allows us to recover approximately 56% of exogenously added esculetin. Plasma was assayed directly for esculetin after precipitating protein with 5% TCA (1:1 v/v). Tail skins were homogenized in 10 ml of 0.1N NaOH after which 10ml of 5% TCA was added. The homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was removed and the pellet re-extracted. The two supernatants were combined, lyophilized, dissolved in 1ml distilled water, microfuged and analyzed via HPLC. Standard curves are prepared from plasma spiked with a known quantity of esculetin and 1:1 (v/v) of 5% TCA. The HPLC method utilized for detecting esculetin was a linear 20-80% methanol gradient over 20 minutes using optical detection at 330nm on a RP C-18

column. This method gave satisfactory results down to 50-100 ng esculetin. We have determined that 500mg/kg esculetin is lethal to 100% of mice injected IP. At 100mg/kg IP esculetin the following plasma and tail skin esculetin levels are obtained are given in Table I.

Table I Skin and Blood Plasma Esculetin Levels

Time* (min)	Tail Skin (ug Total Content)	Blood Plasma (ug/ml)
15	7.38	39.4
30	11.82	97.0
90	-----	24.1
180	3.30	00.0

*time post-injection of 100mg/kg esculetin

The data indicated that esculetin was rapidly excreted from the body. This can be seen visually by urine color (dark yellow/orange) during the first 60min after esculetin injection. Peak plasma concentrations are only 97ug/ml plasma at 30 minutes post-injection, and tail levels are even lower. It is doubtful that a one time dose of esculetin can be an effective chemoprophylactic agent.

b) Tracking of [75Se] Labelled Cercariae in Untreated Mice. See #4 above.

c) The role of esculetin as a prophylactic inhibitor of cercarial penetration. Esculetin (100mg/kg) was injected IP into 18 mice. Five mice were exposed to cercariae immediately after injection, five mice were exposed to cercariae 45mins after esculetin injection and the remaining mice were sacrificed at 15, 30, 90 & 180 minutes after esculetin injection to determine blood and tail skin esculetin levels. In addition 5 mice were given an IP injection of esculetin vehicle and exposed to cercariae via the tail route for 1 hr. The results are given in Table II.

A very slight reduction (10%) in the number of cercariae that migrated to the lungs 7 days post-exposure was noted only for those mice exposed immediately after a 100mg/kg IP dose of esculetin. Since the numbers of cercariae remaining in the tails after 7 days were the same among all 3 groups and esculetin is very short lived in vivo, we concluded that the

Table II Effect of Esculetin *in vivo*

Cercarial Exposure (mins after esculetin injection)		%Cercariae Recovered (7 days p.i.)	
100 mg/kg esculetin	0-60mins	tails	17.5 ± 3.05
		lungs	59.2 ± 8.99
100 mg/kg esculetin	45-105mins	tails	18.2 ± 2.67
		lungs	69.3 ± 2.69
Controls		tails	18.5 ± 5.58
		lungs	68.2 ± 0.98

cercarial reduction occurred during penetration. Blood levels of esculetin during the 1st hour after an IP dose of 100mg/kg reached a high of ~97ug/ml. The entire tail skin; however, has only ~12ug (probably in skin capillary beds). By comparison, our agar:gel penetration plates gave significant inhibition of cercarial penetration and transformation at 1mM esculetin, a concentration of ~174ug/ml. Thus, skin levels were 15x lower and blood levels just under 2x lower than esculetin concentrations that were effective *in vitro*. In addition, when mice were exposed to 200mg/kg esculetin IP using the above design, no significant differences between experimental and control mice were found. Hence, we believe the slight reduction shown at 100mg/kg was not biologically significant. Several experiments were also undertaken using a time course of 100mg/kg and 50mg/kg esculetin given every 1 1/2 hr IP x 4. Due to the toxicity of the vehicle (esculetin is not soluble in saline) these experiments were inconclusive. Given the short half-life of esculetin (< 90min) we do not believe that esculetin has any practical application as a chemoprophylactic agent; hence we discontinued investigating its effect *in vivo*. We decided to screen several other potential drugs that are either more potent lipoxygenase inhibitors or have more favorable pharmacokinetics.

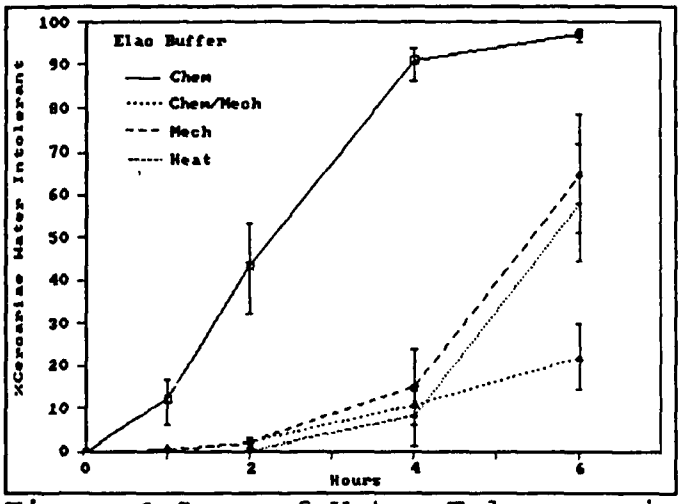

Figure 1 Loss of Water Tolerance in Cercariae Exposed to Various Transformation Methods

6. The Biochemical Mechanisms Involved in Cercarial Transformation.

We finished a series of experiments investigating various cercarial transformation methods with respect to loss of water tolerance, tail loss, eicosanoid production, ultrastructure and RNA, DNA and protein synthesis. Four transformation methods have been utilized in this study: chemical stimulation by 3mM Linoleate, mechanical shearing of tails, chemical/mechanical combination and incubation in buffer. In addition, we have repeated these studies with both ELAC and Saline buffers. Cercariae were analyzed for water tolerance and eicosanoid production at 1, 2, 4 and 6 hrs, while ultrastructure was examined after 1 and 4 hrs of incubation. Water tolerance was assayed by the use of trypan blue (dead cercariae stain dark blue, whereas living cercariae are light blue to clear in color). The results were surprising. Overall, ELAC was a much better buffer than Saline for inducing cercarial transformation as measured via loss of water tolerance. As seen in Figure 1, only those cercariae stimulated chemically lost their tolerance to fresh water after 6 hrs of incubation at 37C (97% ± 1.50, n=8). Mechanically transformed cercariae (ELAC) and those incubated in ELAC at 37C had highly variable transformation rates (=loss of water tolerance) reaching 64.9% ± 13.48 and 58.1% ± 13.54 after 6 hrs respectively. After 4 hours of incubation both mechanical and incubated cercariae had less than a 15% loss of water tolerance. In addition, only chemical transformation yielded the highest rate of eicosanoid production (Figure 2). In fact, mechanical/chemical transformation, which should have given identical eicosanoid production given the presence of linoleate as a substrate, gave significantly less production. We believe this indicates that mechanical transformation methods result in damaged schistosomulae (see below) Data for cercarial/schistosomular EM

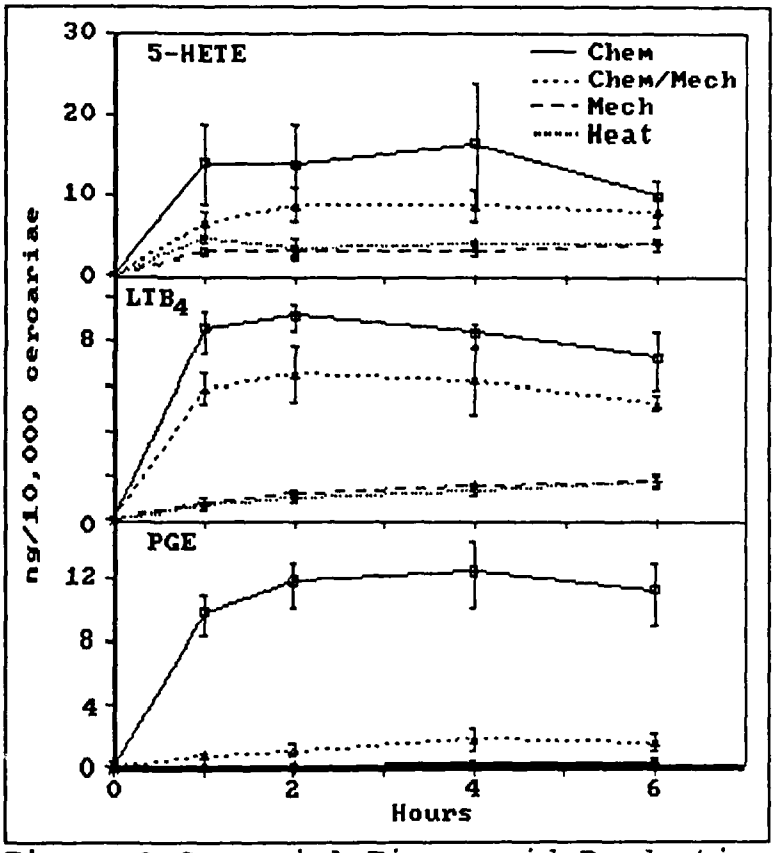

Figure 2 Cercarial Eicosanoid Production After Undergoing Various Transformation Methods

ultrastructural characteristics using various transformation methods are given in Table III.

Table III Schistosomulae EM Characteristics Using Various Transformation Methods

Transformation Method (4hrs incubation)	EM			
	Glycocalyx	Surface Membrane	Nuclear Condition	Granule Migration
3mM Linoleate	A	D	H	++
Heat	I	D	H	+
Mechanical	A	D	H	+
3mM Linoleate + Mechanical	A	D	H	++

A = Absent or reduced I = Intermediate P = Present
D = Heptalaminate membrane S = Trilaminate membrane
H = Heterochromatic E = Euchromatic
(+) = relative degree of cyton granule involvement
(-) = no cyton granules

Our experiments indicate that ultrastructural changes are not good indicators of cercarial transformation, since all transformation methods gave similar ultrastructural changes even though biochemical changes were vastly different. However, Stirewalt reported (Exp. Parasitol. 56:358-368, 1983) that the change from heterochromatic to euchromatic nuclei is a sign of cercarial to schistosomular transformation. We were concerned that none of the transformation methods we utilized produced euchromatic nuclei after 4 hrs of incubation, especially since Stirewalt stated that this change occurred within 1 hr <u>in vivo</u>. Thus we focused our attention on obtaining EMs of schistosomulae <u>in vivo</u> at 1, 4 and 8 hrs after penetrating mouse ear skin. The EMs indicated that none of the schistosomulae examined had euchromatic nuclei. After completing this work we learned that a special staining technique was needed to observe euchromatin in cercariae. Repeating some of the EM work with the new staining technique again showed no euchromatin in transformed cercariae, even though we personally communicated with Dr. Cousin who did the EM work in the paper cited above. In fact, some cercariae which were obviously damaged, showed euchromatic nuclei on staining. Euchromatic nuclei were not seen in chemically transformed cercariae at the end of 4 hrs. Thus, our results differ from those of Stirewalt, This discrepancy may be explained by methodological differences between our lab and

Stirewalt's. Stirewalt allowed cercariae to penetrate ear skin for 1 hr and then collected schistosomulae by cutting and mincing the ear skin. The schistosomulae gathered using such a technique may be damaged, and dead or dying cells tend to show euchromatic nuclei in EM. We exposed cercariae to ear skin, fixed the schistosomulae in situ, and then sectioned ear skin slices looking for schistosomulae. Thus, we believe euchromatic nuclei are not a good indication of schistosomular transformation, but rather a sign of damaged cercariae or schistosomulae. These findings also have bearing on the biochemical data presented below since the change from heterochromatic to euchromatic nuclei is supposed to be an indication of DNA activation. Our biochemical data indicated that an increase in DNA synthesis does not occur during the early stages of transformation. We currently believe that the observation of euchromatic nuclei in transformed cercariae may be an EM artifact.

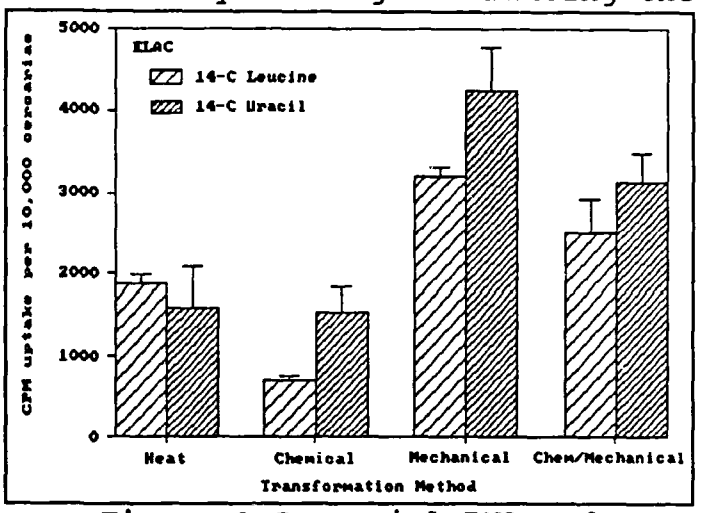

Figure 3 Cercarial RNA and Protein Synthesis and Various Transformation Methods

In addition to ultrastructural studies, we analyzed RNA, DNA and protein synthesis. RNA synthesis was evaluated using ^{14}C-uracil or ^{14}C-orotic acid as precursors, DNA synthesis was analyzed using ^{14}C-thymidine as a precursor, and protein synthesis was measured using ^{14}C-leucine as a precursor (Figure 3). No significant amount of DNA synthesis or orotic acid uptake occurred in any experiment group. However, the ELAC groups did show some uptake of thymidine that was not incorporated into DNA (Figure 4), demonstrating that the cercarial membrane was permeable to the pyrimidine and that thymidine was presumably being incorporated into other compounds (proteins?). The inability of cercariae to use orotic acid as a substrate for RNA synthesis may be due to the lack of enzymes necessary to convert

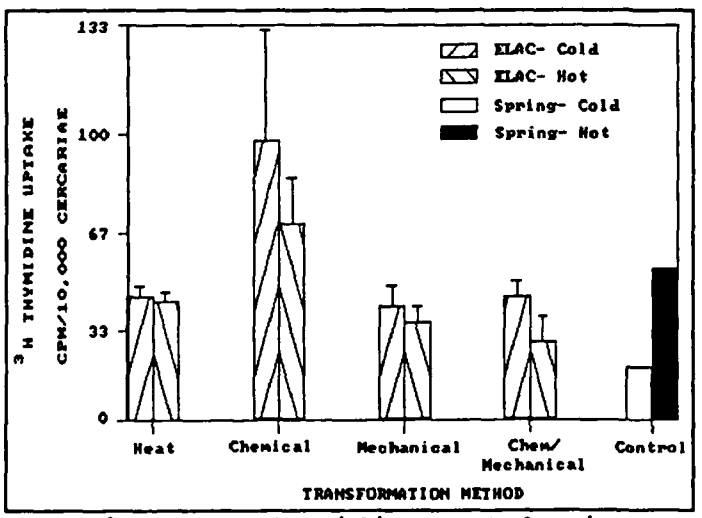

Figure 4 Thymidine Uptake in Cercariae Undergoing Various Transformation Methods

orotic acid to a pyrimidine such as uracil or the impermeability of the cercarial membrane to a highly charged molecule such as orotate. We have found increased incorporation of ^{14}C-uracil in those cercariae that were mechanically or mechanical/chemically transformed when compared to the other transformation methods. This increase strongly suggests that any mechanical means of transformation may damage the organism. We arrive at this conclusion because chemically transformed cercariae have a double membrane (and all other ultrastructural characteristics of schistosomulae), yet do not have high levels of uracil or leucine uptake. Thus the levels of protein synthesis seen in mechanically transformed cercariae are NOT related to membrane turnover or other cell processes reflected in ultrastructure. In fact, these results lend support to the theory that the cyton granules are prepackaged membranes and that cercariae are already "primed" to transform. Given these facts, biological mediators, such as eicosanoids, may be even more important in the transformation process than was previously anticipated. Thus, understanding the role of eicosanoids in the transformation process becomes critical for the rational development of prophylactic agents.

The results of our studies cause us to question the experimental results, especially immunological data, reported in much of the literature published to date, collected utilizing mechanically transformed cercariae. In addition, these data show that ultrastructural changes are not good indicators of cercarial transformation, since all transformation methods gave similar ultrastructural changes even though biochemical changes were vastly different.

7. <u>The Role of Skin Eicosanoid Production in Cercarial Penetration.</u>

We have completed a series of experiments examining the role of skin eicosanoid production in relation to successful schistosome penetration and migration. Several strains of mice that have been reported in the literature to have varying levels of skin eicosanoids and immunological parameters were used as models in these studies. Cercariae labelled with [^{75}Se] were used to track skin penetration and migration in SENCAR, ICR, NMRI, A/J, C3H, C57Bl, Asebic, and BALB/c mice and SD-rats. NMRI mice have been reported to have decreased levels of lipoxygenase products, SENCAR mice have been reported to have elevated levels of lipoxygenase products and ICR mice were used as normal controls. The results obtained clearly showed a difference in both penetration and migration rates between strains and correlated these parameters with host skin eicosanoid production.

Tail skin eicosanoids were measured as follows: briefly, tail skin was homogenized in 0.1M sodium phosphate buffer, centrifuged to remove debris, and the supernatant was collected. The protein homogenate was diluted to 1mg protein/ml, and 3mM linoleate (1μCi ^{14}C-U-linoleate/ml) was added as a substrate for eicosanoid synthesis. After 10, 30 and 60 mins of incubation at 37C, aliquots were

removed, extracted for eicosanoids and subjected to HPLC analysis. Each sample was replicated 5 times. Total eicosanoid production and PG, LT and HETE synthesis were measured by counting DPS recovered between retention times of 7-70 min, 7-24 min, 24-50 min and 50-70 mins respectively.

Cercarial penetration and migration in each strain was measured using [^{75}Se] labelled cercariae and are presented in Figure 5 & 6 respectively. The correlations between tail skin HETE production and tail skin cercarial penetration are presented for 10 and 60 mins as a plot of %penetration vs eicosanoid production (DPS) in Figure 7. When all nine strains were evaluated neither skin PG nor LT production was strongly correlated to cercarial penetration; however, seven of the strains investigated had strong LT vs skin penetration correlations. Tail skin eicosanoid production at 30 mins appears to be a transitory period involving no strong correlations between penetration and HETEs, LTs or PGs and hence are not presented. These data are in agreement with the correlations we have published between cercarial eicosanoid production and penetration of an agar:gelatin substrate (J. Parasitol. 72:397-404, 1986). In addition, a graph of skin HETE production vs cercarial penetration show a definite bell-shaped curve, indicating a certain amount of skin eicosanoid enhances cercarial penetration, but levels above and below the curve maximum actually inhibit penetration.

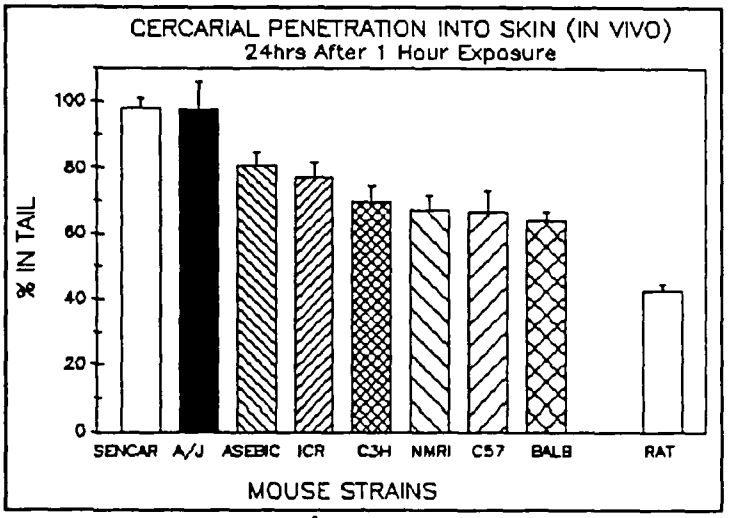

Figure 5

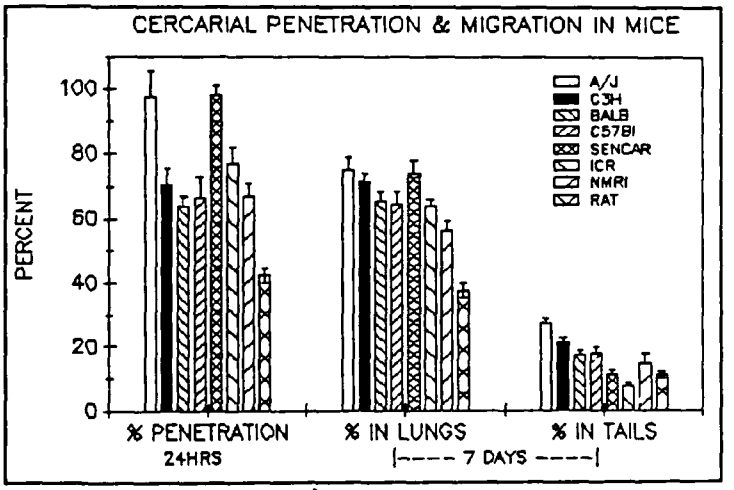

Figure 6

Particularly interesting was the inclusion of the asebic mouse strain in this study. The asebic mouse has no sebaceous glands, thus penetration rates in this model allowed us to evaluate the

role of sebaceous gland secretions in cercarial penetration. Cercarial penetration rates for asebic mice were normal when compared to the ICR strain. Thus, the absence of sebaceous glands, as well as the substances secreted by these glands, do not appear to influence cercarial penetration. Thus we have begun to further define a non-immunological, biochemical basis for cercarial penetration involving host skin eicosanoid production.

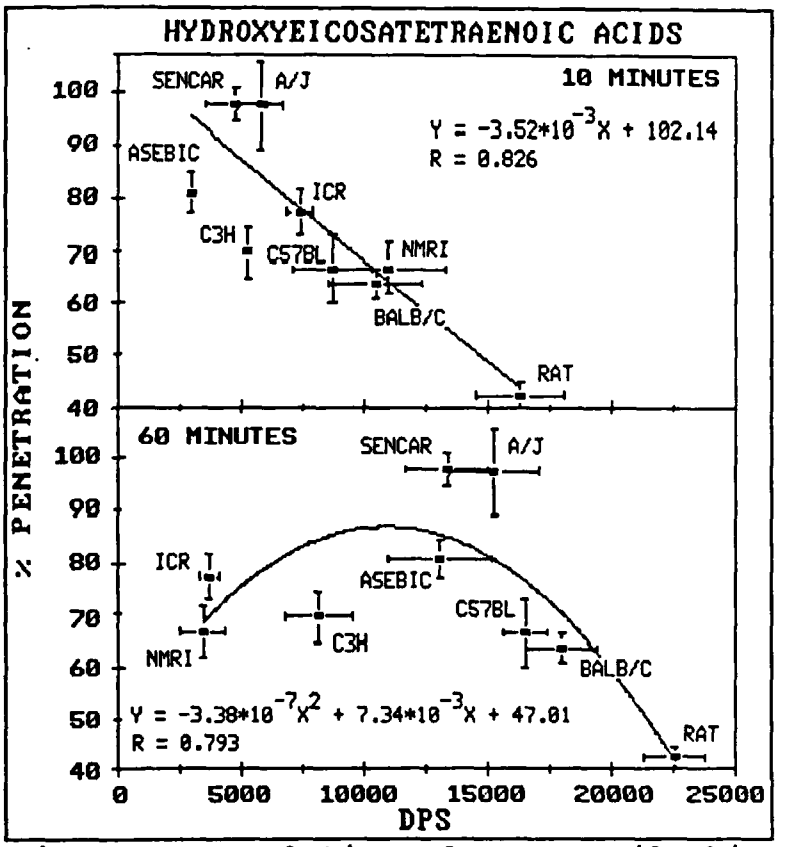

Figure 7 Correlation of Mouse Tail Skin HETE production with Cercarial Penetration Rates in Various Mouse Strains

8. Drug Screening of Various Eicosanoid Inhibitors.

We screened, in vitro, numerous compounds that have been reported to be either potent lipoxygenase inhibitors or to have some anti-schistosomule activity. Screening was done by measuring cercarial stimulation by linoleate. Drugs that showed promise in vitro were examined in vivo using [^{75}Se] labelled cercariae. Drugs that we have screened are recorded in Table IV.

Caffeic acid has been reported in the literature as a slightly more potent lipoxygenase inhibitor than esculetin. However, our in vitro screening has shown it to be ineffective in inhibiting or suppressing cercarial stimulation by linoleic acid. On the other hand, Ketoconazole was very effective in vitro and has recently been reported to be a specific inhibitor of 5-lipoxygenase. Since we have shown that 5-lipoxygenase products, particularly LTB_4, are involved in cercarial penetration, we thought that the effect of ketoconazole in vivo would be particularly worth investigating. In addition, ketoconazole is already in use as an antifungal agent with known pharmacokinetics. PZQ was also very effect in inhibiting cercarial response to linoleate. This drug is currently the DOC in S. mansoni infections. Thus, the possibility of using PZQ as a prophylactic agent was very attractive.

Based on the results of these drug screening studies we decided

Table IV *In Vitro* Screening of Lipoxygenase Inhibitors

Drug	Inhibition Mechanism	pH	mM	Inhibition
Caffeic acid	lipoxygenase	7.0	1	No effect
			10	No effect
			1	No effect
Ketoconazole	5-lipoxygenase	7.0	0.1	94%
			0.001	91%
			0.0001	31%
Amosconate	?	7.0	0.1	100%
			0.01	62%
Imidazole	TxB inhibitor	7.0	1.0	No Effect
NDGA	Lipoxygenase	7.0	0.1	100%
			1.6	71%
			1.7	29%
Praziquantel	?	7.0	0.00001	71%
Propylgallate	Lipoxygenase	7.0	1.0	55%
C-Retinoate	Lipoxygenase	7.0	1.0	No Effect
T-Retinoate	Lipoxygenase	7.0	1.0	No Effect
Sulfasalazine	Lipoxygenase	7.0	1.0	100%
		1.8	0.1	63%
		1.9	0.01	No Effect

to continue investigating, *in vivo*, the following drugs: praziquantel (PZQ), ketoconazole (KETO), and sulfasalazine. Even though NDGA had significant effect on cercarial response to linoleate it was not investigated because recent reports in the literature state that it is not an effective inhibitor of lipoxygenase *in vivo* due to its short serum half-life.

Since both KETO and PZQ were shown to be extremely effective against cercarial stimulation by linoleate *in vitro*, we began screening PZQ and KETO derivatives in the hope that a more effective conformation of these drugs would be found. Screening was done by measuring cercarial stimulation by linoleate. Those drugs that proved to be at least as effective as the parent compound would be screened *in vivo*. The results for PZQ derivatives are given in Table V.

Table V In Vitro Screening of Praziquantel Derivatives

Drug	Derivative of	M	%Inhibition
PZQ	---	10^{-7}	100%
BL21440	PZQ	10^{-5}	25%
		10^{-7}	9%
BL21459	PZQ	10^{-5}	100%
		10^{-6}	56%
		10^{-7}	25%
BL21468	PZQ	10^{-5}	75%
		10^{-6}	30%
		10^{-7}	9%
BL21646	PZQ	10^{-5}	90%
		10^{-6}	0%

Figures 8 and 9 give the time course of PZQ and its derivatives in relation to their ability to inhibit cercarial stimulation by linoleate. As can be seen, none of the PZQ derivatives were as effective as PZQ; thus, we did not test any of these derivatives in vivo.

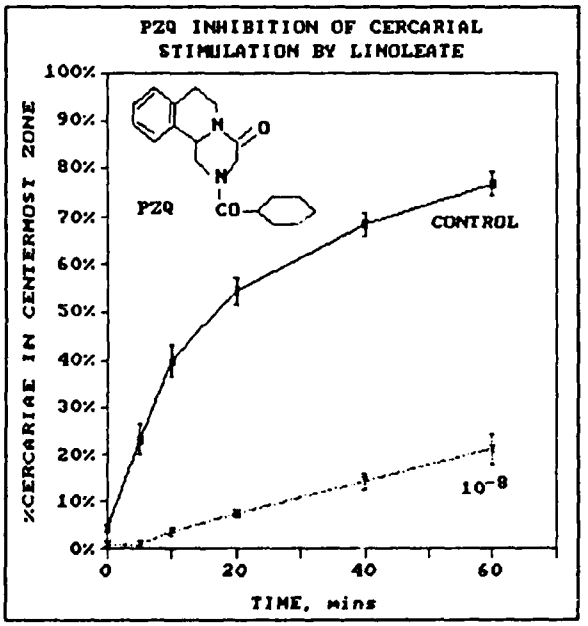

Figure 8

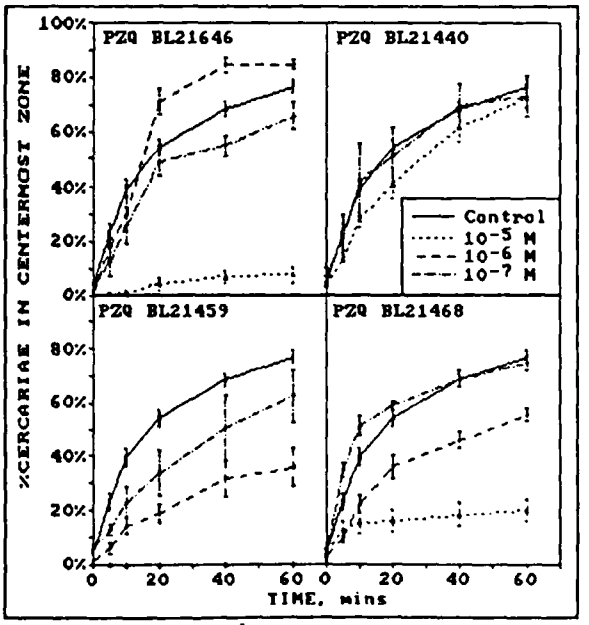

Figure 9

Clearly several drugs as effective or more effective than ketoconazole exist. We investigated the parent compound, ketoconazole, in vivo in order to determine whether this class of drugs was worth investigating. The results of these studies (see #10 below) were disappointing. Thus, these derivatives were not investigated further even though BL28430 might warrant future studies.

In cooperation with Dr. J. Hjelle, University Illinois College of Medicine at Peoria, we examined the effect of three 2-amino-4,5-diphenylthiazole (DPT) derivatives on cercarial eicosanoid production. These compounds represent a new class of eicosanoid inhibitors and thus may be useful in developing possible prophylactic agents. The compounds tested were: 2-amino-4,5-diphenylthiazole (DPT); bis, parahydroxylated, 4,5 phenyl of DPT (diphenol); para-hydroxylated, 5-phenol group of DPT (phenol I); para-hydroxylated, 4 phenyl group of DPT (phenol II). DPT, Phenol I, and Phenol II were strong eicosanoid inhibitors; however, these drugs are available only in small quantities and thus in vitro studies could not be undertake. Given the nature of these compounds, we suggest that future studies screen these drugs in vivo when adequate quantities become available.

Table VI In Vitro Screening of Ketoconazole Derivatives

# DERIVATIVE	KETOCONAZOLE	CONCENTRATION (M)		
	Ketoconazole	10^{-4}*	10^{-5}*	10^{-6}*
1	BL40614	10^{-4}		
2	BL40632	10^{-4}*	10^{-5}	
3	BL40623	10^{-4}*	10^{-5}	
4	BL28136	10^{-4}	10^{-5}	10^{-6}
5	BL28145	10^{-4}	10^{-5}	10^{-6}
6	BL28154	10^{-4}	10^{-5}	10^{-6}
7	BL28163	10^{-4}		
8	BL28172	10^{-4}	10^{-5}	
9	BL28181	10^{-4}	10^{-5}	
10	BL28193	10^{-4}	10^{-5}	
11	BL28207	10^{-4}	10^{-5}	
12	BL28216	10^{-4}		
13	BL28225	10^{-4}		
14	BL28234	10^{-4}*	10^{-5}	
15	BL28243	10^{-4}	10^{-5}	
16	BL28252	10^{-4}*	10^{-5}	
17	BL28261	10^{-4}*	10^{-5}	
18	BL28270	10^{-4}*	10^{-5}	10^{-6}
19	BL28289	10^{-4}		
20	BL28298	10^{-4}	10^{-5}	
21	BL28369	10^{-4}		
22	BL28378	10^{-4}	10^{-5}	
23	BL28387	10^{-4}*	10^{-5}	
24	BL28396	10^{-4}*	10^{-5}	
25	BL28403	10^{-4}*	10^{-5}	
26	BL28412	10^{-4}*	10^{-5}	
27	BL28430	10^{-4}	10^{-5}*	10^{-6}

*Indicates that the derivative prevented all but 15% or fewer cercariae from getting to the center of the target.

9. Experiments on the Use of an Artificial Skin Membrane to Investigate Cercarial Penetration and Transformation Mechanisms in vitro.

We obtained permission to utilize a patented process for the manufacture of an artificial skin membrane. This membrane is a chitin:keratin, collagen:keratin or a chitin:collagen: keratin polymer. This polymer can be varied in size and thickness. In addition, various substances such as linoleate and/or drugs can be incorporated into the polymer. Preliminary experiments showed that cercariae were not able to penetrate through membranes made using the patented formula. In addition, we experimented with a gelatin membrane described by Clegg for use with bird schistosome species as well as a "living-membrane" composed of human keratinocytes.

Our goal was to develop an artificial transformation method that can result in true schistosomes and have a more "natural" method to test drugs in vitro then our current drug screening test affords. The cell type used for these experiments was human epidermal keratinocytes purchased from Clonetics Corporation. The following substrates were analyzed as a basis for an artificial cell membrane:

a) The 'Widra' membrane. Cercariae were not able to penetrate through membranes made using the patented formula. In addition we have tested many different thicknesses, compositions and surface pH values of this membrane with little success. The maximum penetration levels obtained have averaged ~40%. However, the results obtained have not been consistent on a day to day basis. Given the time spent on developing this membrane we did not think it was fruitful to continue pursuing development of this membrane.

b) Collagen Type I Coating & Ketatinocytes. Collagen type 1 was used to coat a 60mm petri dish and overlaid with 2.0×10^5 cells. This method did not form a strong membrane to assay cercarial penetration and stimulation.

c) Boyce Membrane & Keratinocytes. Artificial collagen membranes were obtained from Dr. Boyce and overlaid with either 2.0×10^5, 12.15×10^6, 10×10^6, or 12.75×10^6 cells. The first few membranes were lost due to contamination of the collagen membrane. Once the contamination problem was solved cells were noted to attach to the membrane but did not form a solid sheet over the matrix. When cercariae were added to these membranes, they did not penetrate the substrate itself, but they were attracted to and penetrated the membrane where keratinocytes had attached. Several lost their tails during the process. However, this membrane was not useful since it is very fibrous and does not allow good cell adherence or assay for cercarial penetration and stimulation.

d) Bell Membrane & Keratinocytes. Dermal equivalents were prepared as per Dr. Bell with 2.0×10^5 cells. This collagen (Type I) based dermal equivalent would not gel when formulated according to the published report. We tried modifying this membrane with 5% or 10% gelatin, but it still would not form a solid gel. Contact with Dr Bell did not prove useful; thus this membrane was not pursued.

e) Agar-gelatin & Keratinocytes. Agar gelatin membranes (20%) was overlaid with 3.35×10^6 cells. Keratinocytes did not attach to this substrate and died within a short period of time. In addition, this gel tended to dissolve during the incubation process.

f) Widra membranes & Keratinocytes. 'Widra' membranes (chitosan) were overlaid with 3.5×10^6 cells. Cell sloughed

off this membrane which proved to be a poor substrate for cell growth and attachment.

g) Collagen Type I Membranes & Keratinocytes. Collagen type I membranes (0.3%) were overlaid with 6.25 x 10^5 cells. This method appears to be the best substrate to date. Cells attach to the gel and cercariae are not stimulated by the collagen itself. Cercariae are readily attracted to the keratinocytes where penetration type responses were observed. We are in the process of quantitating this method. The principle difficulty appears to be the ability to make a consistent collagen matrix. [Note: Cercariae exposed to collagen membranes which have not been plated with keratinocytes do not penetrate the gel matrix.]

(1) Trial #1- Plating membranes with 3.4 x 10^6 cells resulted in cells sloughing off membranes at day 12.

(2) Trial 2- Using 6.2 x 10^6 or 7.3 x 10^6 cells resulted in cells detaching from membranes 6 days after plating.

(3) Trial 3- Membranes plated with 2.85 x 10^5 cells remained in good condition out to 4wks. The gels had clumps of cell colonies with fibroblasts visible in the gel matrix. Cercariae exposed to these gels quickly penetrated cells and gel and were found head down at various depths in the gel. Several were seen to lose their tails. Cercarial penetration was estimated at 70-80%.

(4) Trial 4- Membranes were plated with 2.65 x 10^6 cells with 2 supplemented with 3mM linoleate 24hrs prior to testing. Cells attached to these gels and remained viable for at least 3wks. We attempted to use one of these gels over a cercarial penetration chamber; however, manipulation of the gels caused a loss of cells and the gel was not rigid enough to remain seated over the chamber. When cercariae were exposed to membranes treated with linoleate, they did not attempt to penetrate the gel. Instead they remained on the surface jerking spasmodically and eventually became still, probably due to the stimulatory nature of linoleate. Cercariae penetrated (~20%) the gel that did not contain linoleate, especially where clumps of cells were evident.

(5) Trial 5- Collagen gels were plated with 3.1 x 10^6 cells. Cells started to slough off in clumps 15 days after plating. Cercariae exposed to these gels gave penetration rates of 75 and 48%. After cercarial penetration, gels were re-incubated for 19hrs at room temperature. Most cercariae were still alive and many had lost their tails.

h). Matrigel & Keratinocytes. Matrigel (Collaborative Research Inc.), a commercial basement membrane preparation, was used

as a substrate for keratinocyte attachment. Matrigel membranes of various thickness plated with 4 x 10^5, 2.94 x 10^6 or 5.88 x 10^6 cells were investigated. Cells did not attach well. They clumped together in a rope-like aggregation and then started peeling off.

These studies indicated that collagen membranes showed the most promise as a substrate for a "living-cell" membrane; however, the membranes produced were inconsistent in texture and composition. Some membranes allow attachment and growth of cells, whereas others prepared identically cause poor cell growth. We suggest that future studies explore commercial sources of collagen preparations which may give a more consistent membrane preparation. Since cercariae do not penetrate the collagen itself, but are attracted to the keratinocytes and penetrate the gel only when these cells are available, we believe these membrane could prove ideal for studying cercarial penetration as well as screening potential prophylactic agents. Unfortunately, we were not able to satisfactorily produce such a membrane.

10. <u>The Use of Ketoconazole as a Prophylactic Agent (24hr tails & 7 day lungs).</u>

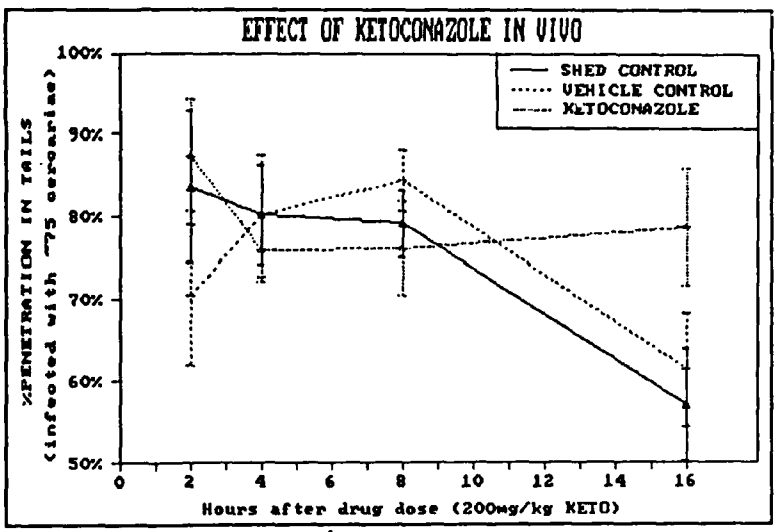

Figure 10

We finished evaluating the effect of ketoconazole, a known inhibitor of 5-lipoxygenase and an FDA approved dermal antifungal agent, as a potential prophylactic agent. A literature search of the pharmacokinetics of this drug indicated that the drug was found on the skin surface and hair of guinea pigs as early as 8 hrs after ingestion of a single oral dose of 40mg/kg. Since ketoconazole is well tolerated, we gave mice a single oral dose 200mg/kg, a relatively high concentration of drug. Cercarial penetration as well as schistosomular migration was evaluated at 2, 4, 8 and 16 hrs after ingestion of 200mg/kg ketoconazole. Cercarial penetration and schistosomular migration was evaluated via autoradiography of tails at 24hrs and lungs 7 days after exposure to ~75 (tails) or ~150 (lungs) ^{75}Se radiolabelled cercariae. Appropriate shed controls and vehicle controls (acidified water) were included in the study. Each experimental group contained 12 male ICR mice 4-6wks of age. Figure 10 shows that ketoconazole has no ability to inhibit cercarial penetration; in fact, it may increase penetration at 16hrs.

Schistosomular migration to the lungs was moderately inhibited (~25%) 8 hrs after ketoconazole ingestion (data not shown). Given the high dosage of ketoconazole used in this study, we have concluded that a one-time dosage of ketoconazole has a minimal protective effect on schistosomular migration. While it is possible that multiple dosages may increase protection, we did not explore this avenue.

11. The Effect of PZQ on Cercarial Penetration, Transformation, and Eicosanoid Production in vitro and in vivo.

Since PZQ was such an effective inhibitor of cercarial response to linoleate, we investigated its effect on cercarial eicosanoid production via HPLC (Figure 15a). At pH 6.5 and pH 7.0, 1mM PZQ decreased overall eicosanoid production by ~50%. However, every eicosanoid peak was decreased by the same amount. This suggested that PZQ may not be a specific inhibitor of eicosanoid production and that the overall decrease in eicosanoid synthesis may be the result of an indirect mechanism. However, we have no proof that this is the case.

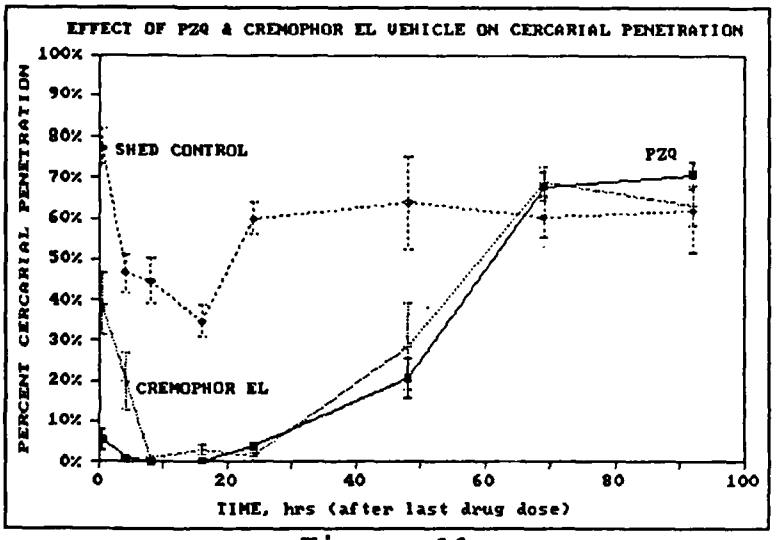

Figure 11

PZQ was investigated as a potential prophylactic agent given its dramatic success in vitro as well as its moderate effect as an eicosanoid inhibitor. Mice were given either 50 or 200 mg/kg PZQ x 4 every 2 hrs using Cremophor El as a vehicle. At the end of the regimen, mice were exposed to either 75 or 150 cercariae labelled with [^{75}Se]. Those mice receiving 75 cercariae were sacrificed at 24 hrs and their tails removed to detect how many cercariae penetrated the skin. Mice receiving 150

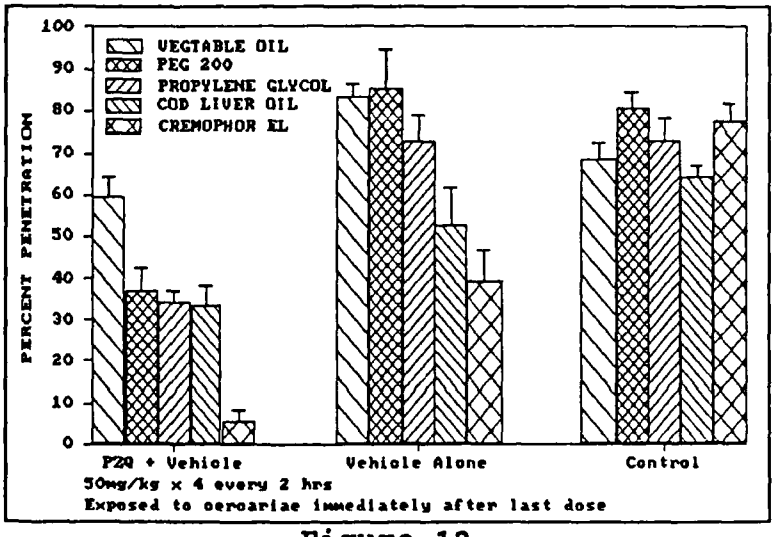

Figure 12

cercariae were sacrificed at 7 days post-infection and their lungs and tails were removed to determine %lung migration rates. No autoradiographic dots could be detected at 24 hrs or 7 days post-exposure in either PZQ dose regimen, while controls had normal levels of penetrating cercariae. Based on these results, a drug regimen of 50mg PZQ every 2 hrs x 4 was selected for all subsequent studies.

A second experimental study was undertaken to examine the effectiveness of PZQ over time. A cercarial exposure time (~75 cercariae/mouse) of 0, 4, 8, 16, 24, 48, 68, and 92 hrs after the last dose of PZQ was selected. Each time period had 5 control mice (no drug or vehicle), 5 vehicle controls (Cremophor El given every 2hrs x 4) and 10 PZQ treated mice (50mg/kg every 2hrs x 4). All mice were sacrificed 24hrs post cercarial-exposure, tails were removed, and tail skins were subjected to autoradiography for 2 weeks. The results of these trials are given in Figure 11. Both PZQ-treated and Cremophor El-treated mice had significantly lower cercarial penetration than controls. In these experiments Cremophor El, in and of itself, had an effect equal to that of PZQ from 8 to 48 hrs. In this case, PZQ gave increased protection over Cremophor El earlier (0-4hrs), but had no real effect 8 hrs after the last drug dose.

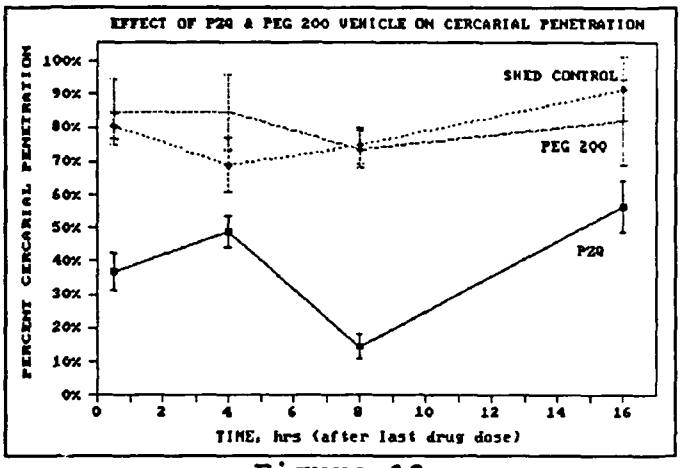

Figure 13

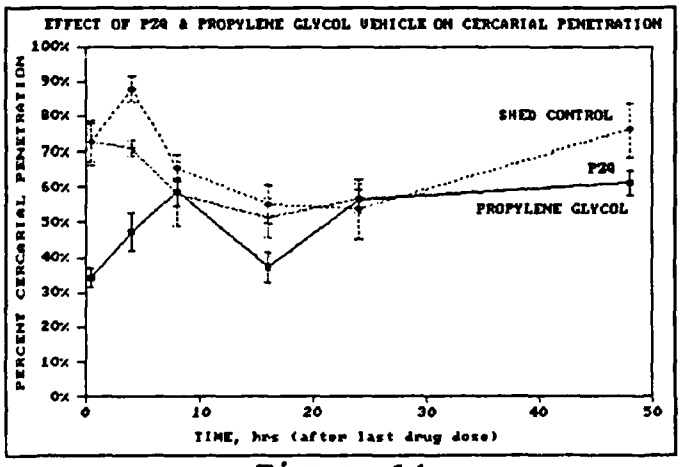

Figure 14

In order to test the role of vehicles on the effectiveness of PZQ, we dissolved PZQ in vegetable oil, 50% PEG 200, 50% propylene glycol, cod liver oil or Cremophor El. Immediately after the last drug dose, 10 mice were exposed to ~75 [^{75}Se] labelled cercariae, 5 were exposed to vehicle only, and 5 were untreated (shed) controls. Mice were sacrificed 24 hrs post cercarial-exposure, tails were removed, and tail skins were subjected to autoradiography for 10 days using Kodak XAR film and Dupont Cronex Lightning-plus intensifying screens. The results of these trials are given in Figure 12. Only vegetable oil proved to be a poor vehicle. PZQ dissolved in PEG

200, propylene glycol or cod liver oil gave equivalent penetration rates, while PZQ in Cremophor El gave the best protection. Cremophor El and to a lesser extent cod liver oil had anti-penetration effects without PZQ being present.

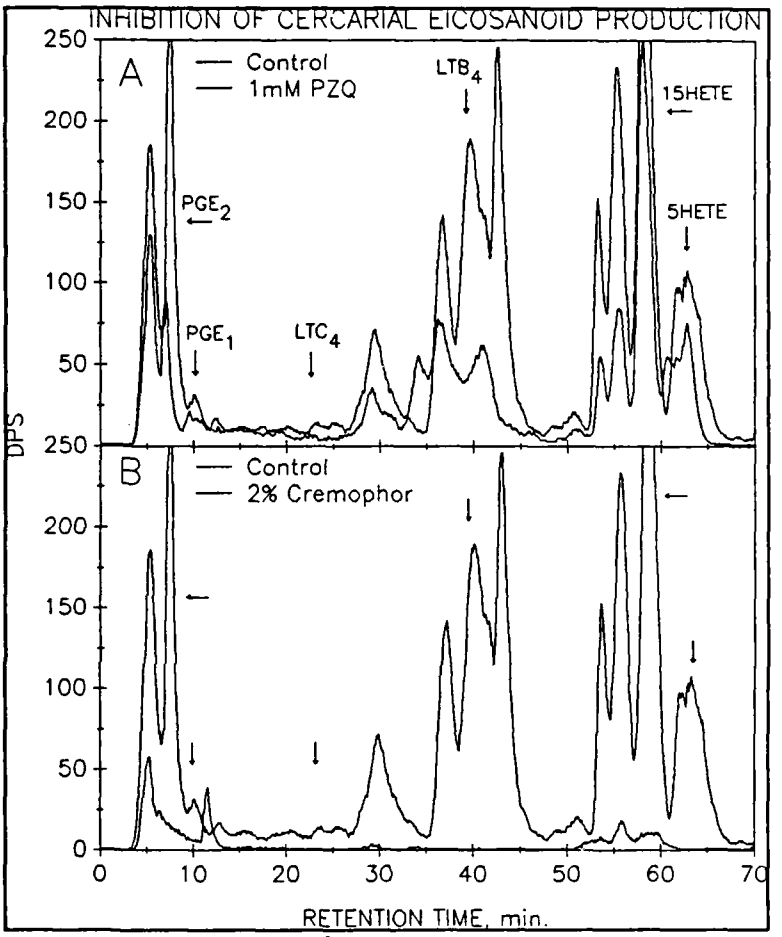

Figure 15

The effectiveness of PZQ over time was then examined using PEG 200 and propylene glycol as vehicles and compared with the results that were obtained using Cremophor El (essentially Castor Oil). PEG 200 as a vehicle for PZQ was examined out to 16 hrs after the last drug dose, while propylene glycol was investigated out to 48 hrs after the last drug dose. Cercarial exposure times (~75 cercariae/mouse) were 0, 4, 8, 16, 24, and 48 hrs after the last dose of PZQ. Each time period had 5 control mice (shed controls, no drug or vehicle), 5 vehicle controls and 10 PZQ treated mice (50mg/kg every 2 hrs x 4 per os). All mice were sacrificed 24 hrs post cercarial-exposure, tails were removed, and tails skins were subjected to autoradiography as detailed above. Figure 13 gives the results using PEG 200 as the vehicle for PZQ, and Figure 14 gives the results using propylene glycol. As can be seen PZQ was effective at times 0, 4 and 16hrs. However, the maximum reduction in cercarial penetration never reached more than 55% at any time period. Clearly this in an unacceptable level of protection.

The drug trials indicate that PZQ may hold promise as a prophylactic; however, its effectiveness is clearly vehicle dependent. The effectiveness of Cremophor El without PZQ suggested that a combination of dietary and chemoprophylactic methods may be worth exploring. Thus we focused our efforts during the last year of this contract on exploring the role of Cremophor El, and derivatives, in vitro and in vivo.

Table VII The Effect of Cremophor El on Cercariae In Vitro

%Cremophor El	Stimulant	%Inhibition	%Stimulation
0%	Linoleate	0%	100%
0%	Cremophor El	0%	0%
1%	Linoleate	47%	0%
2%	Linoleate	36%	0%
4%	Linoleate	50%	0%
0%	Cremophor El over Linoleate*	29%	0%

*We applied Cremophor El over a target of linoleate (normal stimulant) to investigate whether Cremophor El could "mask" the stimulatory effect of linoleate.

12. The Effects of Cremophor El on Cercariae in vitro.

In order to examine whether Cremophor El had any direct effect on cercariae, we measured cercarial stimulation by Cremophor El and the ability of this compound to "mask" the stimulatory effect of linoleate. The analysis of these data is shown in Table VII. Cremophor El can inhibit cercarial response to linoleate; however, at least at the concentrations used (1-4%), this response was NOT dose dependent. Cercariae were not stimulated by Cremophor El alone, nor did it have a strong ability to mask linoleate when applied directly over it.

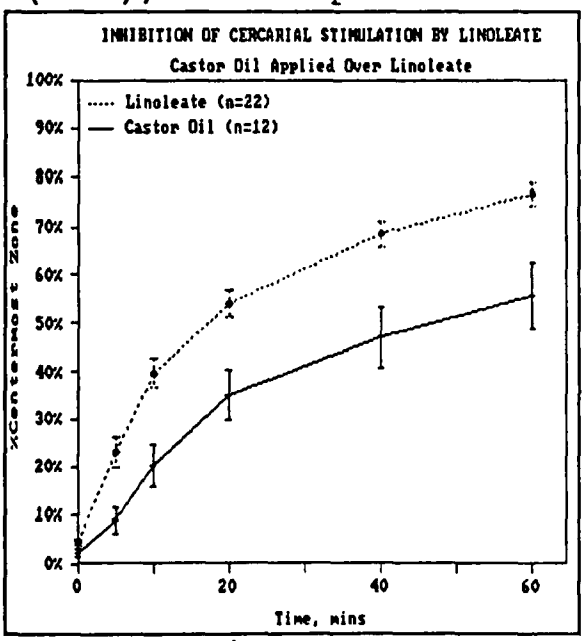

Figure 16

13. Cercarial Stimulation & Inhibition by Castor Oil & Ricinoleic Acid.

Cremophor El is an ethoxylated derivative of Castor Oil, while Castor Oil in return, is composed mainly of ricinoleic acid as a triglyceride. In order to discern any possible role Castor Oil and ricinoleate might have in preventing cercarial penetration we investigated whether these compounds could either stimulate cercarial transformation or whether they could mask cercarial stimulation by linoleate. Our standard "chemotactic" assay using 35mm petri dishes was used employing Castor Oil or ricinoleate as the stimulant. Castor Oil

was not stimulatory (i.e.able to induce transformation events leading to loss of water tolerance) to cercariae (data not shown), nor was it able to completely mask cercarial stimulation by linoleate (~24% reduction, Figure 16). Ricinoleate, however, was as stimulatory as linoleate after 60 min exposure (Figure 17). Since eicosanoids cannot be synthesized using ricinoleate as the substrate, if ricinoleate is the active component of Castor Oil and Cremophor El then it may act by causing cercariae to be stimulated without inducing penetration, thus causing loss of water tolerance and resulting death in fresh water. Ricinoleate treatment does cause skin lipid changes which supports this hypothesis (see #16 below).

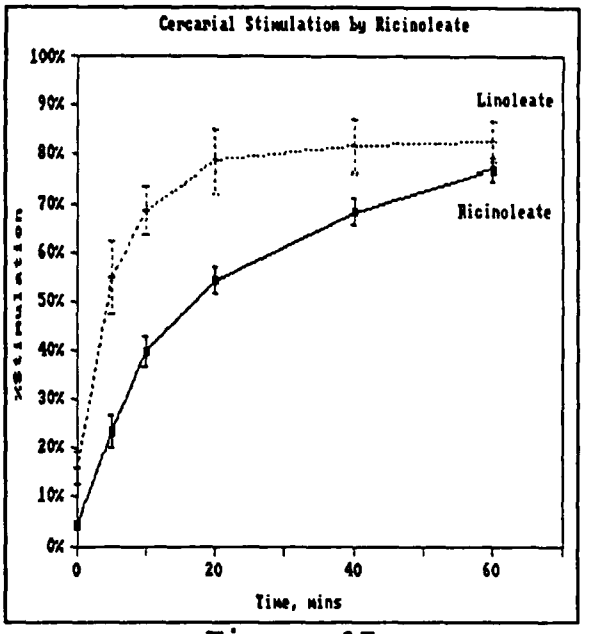

Figure 17

14. Inhibition of Cercarial Eicosanoid Production via Cremophor El, Castor Oil, & Ricinoleate.

We evaluated the effect of Cremophor El, Castor Oil, 80% pure ricinoleate and 99% pure ricinoleate on cercarial eicosanoid production via our HPLC methodology. All incubations contained 3mM linoleate, were for 1hr at 37C in ELAC (pH 7.2) and were repeated 5 times for each condition. Oils were added as a 2% suspension. Figure 15b gives the result of these trials with Cremophor El, which shows dramatic inhibitory effects on cercarial eicosanoid production (100%). Castor Oil had almost no effect on cercarial eicosanoid production, whereas ricinoleate had modest inhibitory activity (data not shown). After analyzing these data, we are concerned that these oils have widely differing solubilities in ELAC, with Cremophor El being the most soluble and Castor Oil the least soluble. Thus, these results with Castor Oil and ricinoleate can only be regarded as TENTATIVE until these experiments can be repeated using sonicated and emulsified ELAC-oil solutions.

15. The Effect of Cremophor El, Castor Oil, & Ricinoleic Acid as Prophylactic Agents.

Since the *in vitro* effects of Castor Oil and Ricinoleate were so promising, as were the *in vivo* effects of Cremophor El, we evaluated the chemoprophylactic effects of Castor Oil and ricinoleic acid as compared to Cremophor El. (Cremophor El is ethoxylated Castor Oil and Castor Oil's major constitute is a triglyceride of ricinoleic acid).

Twelve mice/group were gavaged with 0.3ml/day (9.8g/kg) of either water (controls), Cremophor El, Castor Oil, or 80% pure ricinoleate for 7 days. On the 8th day mice were exposed to ~75 radiolabelled cercariae via the tail route. Twenty-four hrs later all mice were sacrificed and their tails were removed and subjected to autoradiography to determine percent cercarial penetration. Figure 18 details the results of these experiments. Castor Oil gave 99% protection, while Cremophor El and ricinoleate gave 36% and 48% reductions respectively.

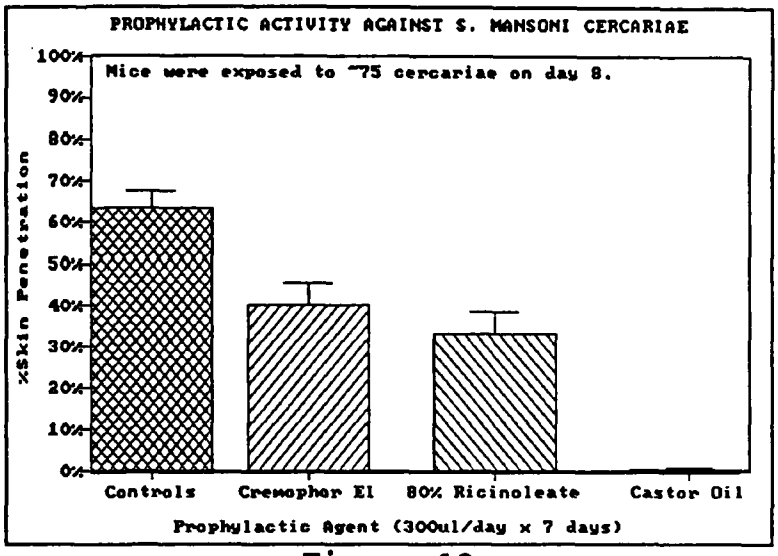

Figure 18

In order to determine the effective dose of Castor Oil we exposed mice, 10 mice/group, to 9.8, 4.9, 2.45 or 1.3 g/kg/day of Castor Oil for 7 days (0.3 to 0.04ml/day). On the 8th day mice were exposed to ~75 cercaria via the tail route and processed as above. Figure 19 details the results of these experiments.

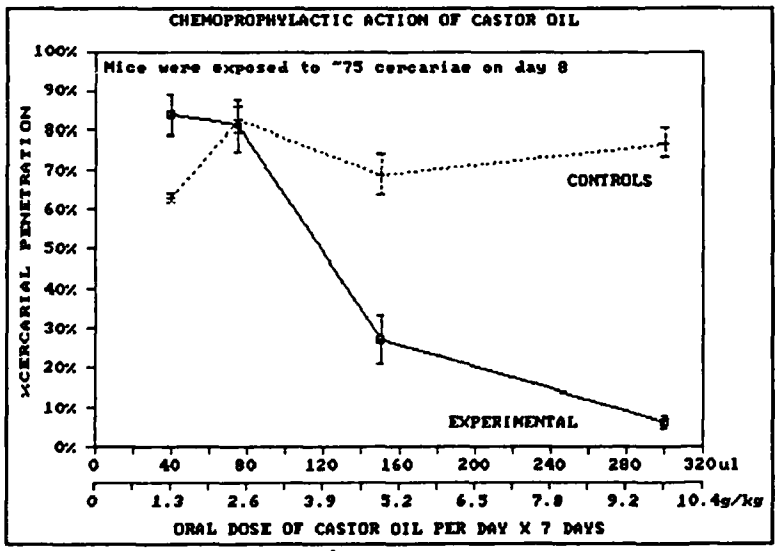

Figure 19

Based on these results and our eicosanoid hypothesis, which states that cercarial eicosanoid production is vital to successful penetration and transformation, we did a complete chemoprophylatic screen on Castor Oil in which we analyzed the time course and duration of the effect of Castor Oil as a protective agent. In these experiments mice were given 9.8g/kg Castor Oil (300ul) per os for 1 to 7 days and then exposed to [75]Se-labelled cercariae anywhere from 2 to 7 days after the last Castor Oil treatment. The complete experimental design is given in Table VIII.

Table VIII Experimental Design for Castor Oil Prophylaxis

Experiment	Drug Duration (days)	Cercarial Exposure (days after last dose)
1	7	1
		3
		5
		7
2	3	1
		3
		5
3	1	1
	3	1
	5	1
	7	1

Figure 20 details the chemoprophylactic effect of giving mice 9.8 g/kg Castor Oil per os for 1 to 7 days and exposing them to cercariae 24 hrs after the last drug dose. Each experimental group had 10 mice and control groups had 5 mice. From this data it appears that some protection can be obtained from a single dose of Castor Oil (only 20% cercarial penetration); however, at least 3 days of treatment is necessary to obtain greater than 90% protection. After 3 days of treatment, no statistical difference between treatment groups was found.

Based on the above results, we examined the decay of the protective effect of Castor Oil after treatment for 3 or 7 days. Ten mice were treated with 9.8 g/kg per day of Castor Oil for 3 or 7 days, while control groups contained 5 mice. They were then exposed to ~75 [75]Se-radiolabelled cercariae at 1, 3, 5 or 7 days post-treatment. Figure 21 shows the decay of the prophylactic effect of 3 days of Castor Oil treatment on cercarial penetration while Figure 22 shows the effects of 7 days of treatment. Interestingly, Castor Oil treatment for 3 days had more shallow decay

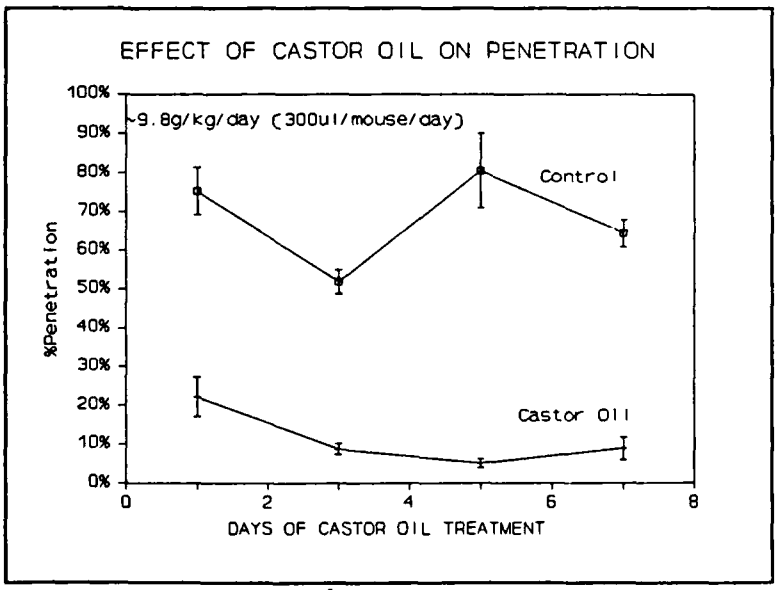

Figure 20

curve than did treatment for 7 days. Seven days post-treatment those mice having received Castor Oil for 3 days still had ~50% protection rates, while those treated with Castor Oil for 7 days were not protected. We postulate that this effect may be due to modification of the surface skin lipid mantle or skin lipid content by Castor Oil treatment. For instance, 3 days of treatment may leave optimal levels of inhibitory substances on the skin surfaces, whereas continued treatment may result in too much or too little skin lipid perturbation (see section #16 for experimental support).

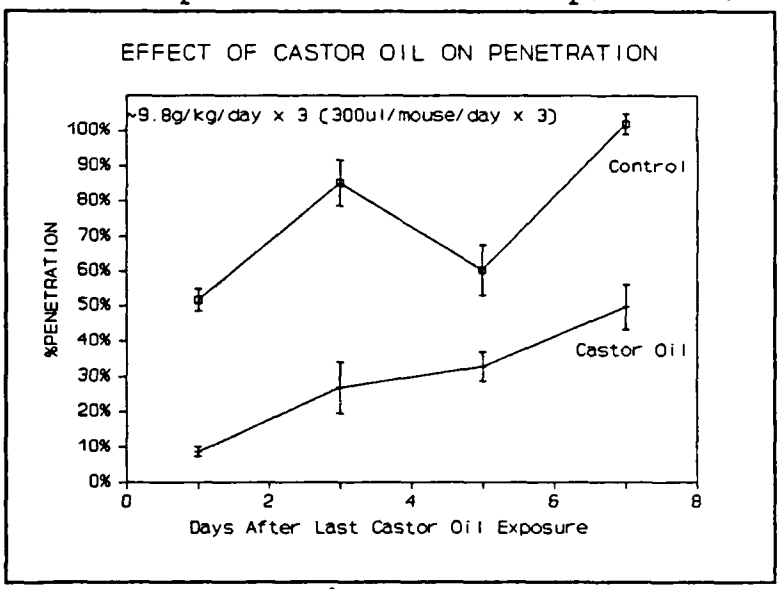

Figure 21

16. <u>Free Fatty Acid Analysis of Skin Surface After Treatment with Cremophor El, Castor Oil & Ricinoleic Acid.</u>

We refined an HPLC method for separating free fatty acids in the C16 to C24 range. This method involved making 2-nitrophenylhydrazide derivatives of the free fatty acids. These derivatives could then be detected optimally at 400nm or 218nm with a 100 fold increase in sensitivity at 218nm. Using these derivatives and detection at 218nm, sensitivities on the order of GC analysis can be obtained. Derivatized fatty acids are injected into a C-8 reverse phase column and eluted with a 82-90% convex gradient (#8 on a Waters 660) of acetonitrile:water for 12mins.

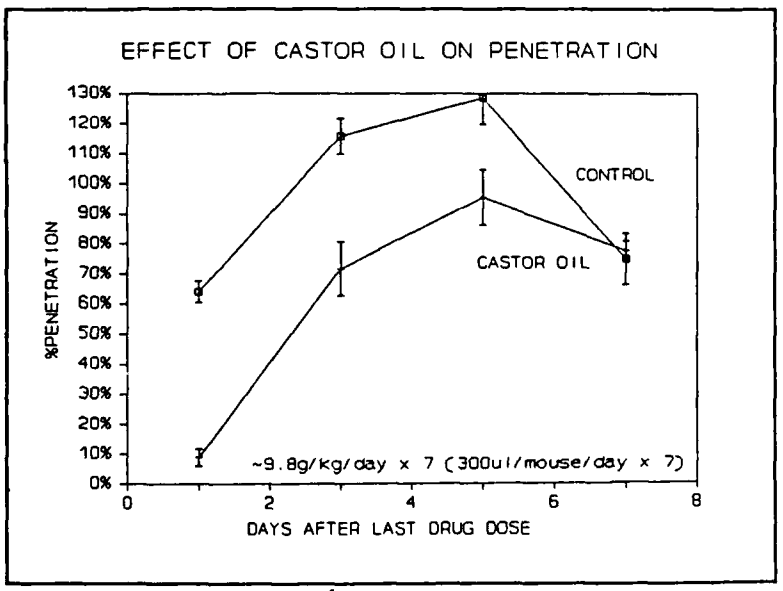

Figure 22

In order to determine if treatment with Castor Oil, Cremophor El or ricinoleate changes the surface free fatty acid composition we undertook the following experiment. Five mice per group were given 9.8g/kg <u>per os</u> (300ul) of Cremophor El, Castor Oil, or ricinoleate

Table IX Summary of Tail Skin Free Fatty Acids Chloroform Extraction of Whole Tail Skin

R.T.	Identification	Diet Supplement (300 ul/day x 7 days) (ug)/mouse			
		Castor Oil	Cremophor El	Ricinoleate	Control
4.29		2.23 ± 0.32	85.16 ± 10.08	71.63 ± 17.31	
4.56		5.82 ± 0.97	111.03 ± 32.87		
4.70	Ricinoleate	34.56 ± 14.00	12.47 ± 3.28	94.42 ± 16.44	89.59 ± 23.11
5.29	Myristoleate	93.31 ± 18.19		1.15 ± NA	4.98 ± NA
6.06				0.89 ± NA	0.80 ± NA
6.60			1.21 ± 0.50	2.03 ± 0.62	
7.07			6.36 ± 0.71	5.80 ± 1.55	1.22 ± NA
7.43	Linolenate	1.45 ± 0.58	1.38 ± 0.16	4.14 ± 2.35	0.95 ± NA
7.80	Docosahexanoate	1.52 ± 0.69		2.64 ± 0.12	
8.33	Arachidonate	8.65 ± 1.28	13.50 ± 1.13	6.10 ± 0.63	11.22 ± 1.90
8.90		8.38 ± 1.44	3.34 ± 0.93	3.48 ± 1.02	8.03 ± 1.02
9.70		3.38 ± 0.82	6.13 ± 0.45	3.24 ± 0.79	4.53 ± 0.26
10.26		2.91 ± 0.50	4.51 ± 1.78	10.96 ± 0.88	8.11 ± 1.11
10.76		11.52 ± 1.58	12.07 ± 1.64		
11.44	Oleate	4.29 ± 0.60	2.50 ± 0.60	3.99 ± 1.31	2.44 ± 0.65
12.12	Petrosilinate	0.85 ± NA	1.22 ± 0.00	±	±
14.51		16.61 ± 2.94	17.72 ± 2.32	18.64 ± 1.16	15.51 ± 1.58

for 7 days. Five control mice were not treated. On day eight all mice were sacrificed, tails were wiped with chloroform:BHT (BHT was added as an anti-oxidant), removed and homogenized in chloroform:BHT. Tail wipes from each group and whole tail homogenates from each group were pooled, and centrifuged, and the chloroform layer was removed and evaporated to dryness under a stream of nitrogen. The residual was taken up in 3ml of chloroform:BHT, put into chloroform-washed glass vials, overlaid with nitrogen, sealed and kept at -20C. Thus 8 vials remained to be analyzed: tail wipes from Cremophor El, Castor Oil, ricinoleate and controls and whole tail homogenates from the same. It should be noted that in these experiments extreme care was taken not to contaminate surfaces with lipid. For example, all glassware was thoroughly washed with detergent and water and rinsed with distilled water followed by 95% ETOH followed by chloroform to make sure no lipids remained on any glass surface.

Free fatty acid analysis from these samples are given in Tables IX and X. We have come to the conclusion that there were no <u>obvious</u> surface free fatty acid changes that appear to give Castor Oil its superior prophylactic effect. Therefore, the study was repeated to see if there were any changes in skin surface lipid classes, i.e. phospholipid, glycolipid, triglyceride, etc. These classes were analyzed via TLC (thin-layer chromatography) on silica gel plates using a 2-stage development system. The first solvent was isopropyl

Table X Summary of Tail Skin Fatty Acid--- Chloroform Tail Wipes

R.T.	Identification	Diet Supplement (300 ul/day x 7 days) (ug)/mouse: Castor Oil	Cremophor El	Ricinoleate	Control
4.08		127.31 ± 40.24	104.24 ± NA	81.58 ± 34.80	145.38 ± 32.06
4.42	Ricinoleate	45.74 ± 3.44	140.76 ± 28.55	62.75 ± 12.31	45.22 ± 9.39
5.55	Myristoleate	0.91 ± NA	0.55 ± NA	1.06 ± NA	
6.38			0.78 ± NA		
6.88			6.56 ± 0.73	3.32 ± 0.12	
7.12	Docosahexanoate	3.26 ± 1.33	2.76 ± 1.47	1.36 ± NA	2.41 ± 0.92
7.66	Palmitoleate		1.93 ± NA	0.39 ± NA	0.09 ± NA
8.03	Arachidonate		9.99 ± 1.12	4.84 ± 0.86	3.53 ± 1.63
8.45	Linoleate	7.20 ± 0.77	3.31 ± 1.69	3.17 ± NA	6.60 ± 0.49
9.43		3.14 ± 0.12	6.56 ± 1.31	5.81 ± 2.16	3.06 ± 0.60
10.02		18.95 ± 2.88	7.17 ± 2.82	9.37 ± 1.40	10.80 ± 1.34
10.90	Oleate	2.53 ± 0.46	1.58 ± NA	0.44 ± NA	2.50 ± 0.11
14.01		20.44 ± 1.52	18.15 ± 2.53	17.33 ± 1.80	17.19 ± 2.49

ether-acetic acid (96:4) followed by petroleum ether-diethyl ether-acetic acid (90:10:1). Skin surface lipids were applied to the plates in a chloroform vehicle from 10 to 50 ul in volume. Chromatography spots were visualized by iodine vapor and identified by co-chromatography of known standards of phospholipid, free fatty acid, fatty acid methyl esters, triglyceride, diglyceride, monoglyceride, cholesterol and cholesterol esters. Spots were quantitated by using a standard method involving sulfuric acid:potassium chromate oxidation as measured by OD readings at 350nm. The results are given in Figure 23. Castor Oil caused moderate increases in free fatty acid, cholesterol esters, and 2 unidentified skin lipids. Ricinoleate treated animals showed dramatic changes in skin lipid content. Phospholipid, mono- and triglyceride, and free fatty acid levels all increased, while cholesterol esters decreased. In addition 2 unidentified skin lipids also increased. These effects are in addition to any lipid changes that may have taken place in epidermal or dermal tissues. Clearly,

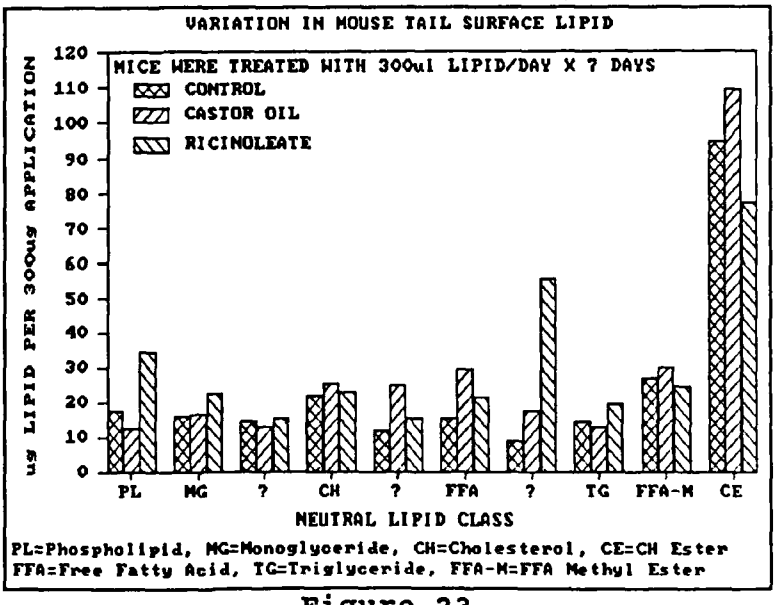

Figure 23

this should be an area of future investigation, especially since #17 below shows that penetration rates can be changed by varying surface lipid composition.

17. Inhibition of Cercarial Penetration by Skin Lipid Changes. In order to determine if skin lipid changes can inhibit or promote cercarial penetration, we topically treated the tail skin of mice with oleate, Cremophor El, or linoleate. Mice were then exposed to ~75 radiolabelled cercariae and percent penetration was calculated 24 hrs later. The results are shown in Figure 24. As can be seen lipid changes on the surface of the tail can cause dramatic changes in cercarial penetration. Interestingly, high linoleate concentrations (an essential fatty acid and eicosanoid precursor) also causes a dramatic decrease in penetration. This is because cercariae are induced to transform upon contact with high linoleate level. Transformation then leads to loss of water tolerance and death. Thus cercariae are killed before having a chance to penetrate skin. Cremophor El may inhibit penetration by virtue of its strong eicosanoid inhibitory effect. These studies indicate that skin surface lipid perturbations can result in inhibition of cercarial penetration.

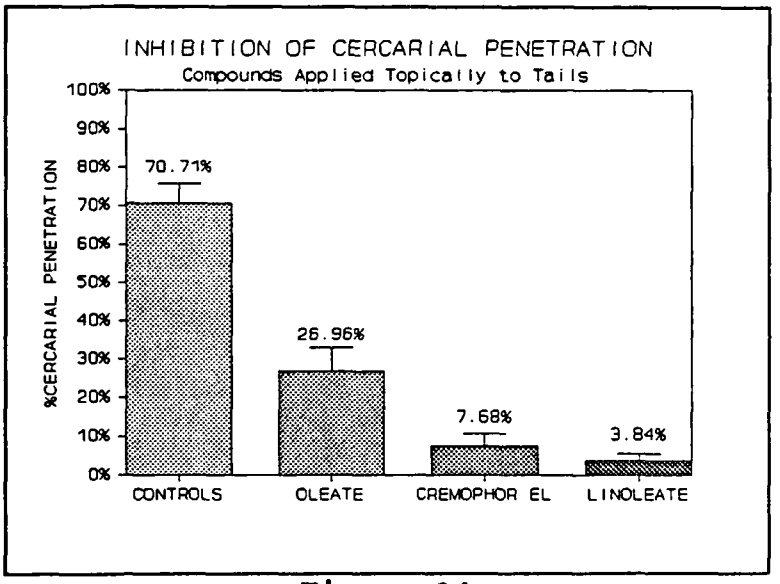

Figure 24

18. In Vivo Effect of 2 Ricinoleate-Like Compounds.

A major component of Castor Oil is ricinoleate as triglyceride. In order to determine whether the triglyceride formulation is important in Castor Oil's prophylactic action, we began to study the dose-time activity responses of free ricinoleic acid. Near the end of this contract our COTR supplied us with a structure report detailing well over 100 compounds having a similar structure to ricinoleate. We were able to obtain two compounds in sufficient quantities and in enough time to evaluate them in vivo before this contract expired. The structures

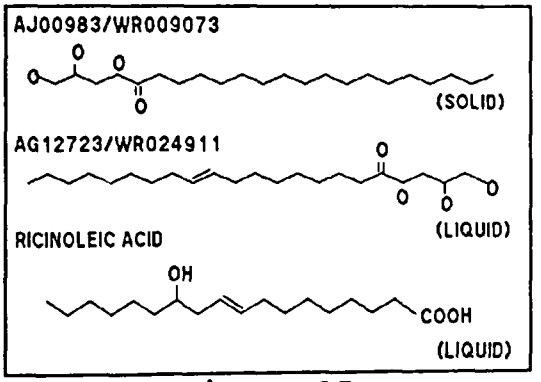

Figure 25

of these compounds are given in Figure 25 and are compared to ricinoleate. We treated 10 mice once per day for 7 days with either AJ00983 (50mg/kg in DMSO) or AG12723 (167ul/mouse). In addition, 8 mice received DMSO only as a control. As was our custom, 5 shed controls for each group were also included in the study. Neither of these drugs were effective in inhibiting cercarial penetration (data not shown). This suggests that highly oxygenated lipid head or tail groups provide little protection. We postulate that an OH group 2 or more carbons away from a double bond is necessary for activity. Also, given the increased prophylactic effect of Castor Oil over ricinoleate, a triglyceride may be necessary for full effect. We suggest the design of such lipid molecules based on the structure of ricinoleate would prove fruitful.

Table XI Comparison of Secreted and Non-Secreted Eicosanoid Production in Schistosoma mansoni Cercariae and Adults

Total Eicosanoid	Location	Cercariae	Adults Male	Adults Females
PG	Supernatants	63 ± 8%	81 ± 3%	89 ± 3%
	Whole Worms	37 ± 8%	19 ± 3%	11 ± 3%
LT	Supernatants	75 ± 6%	78 ± 3%	86 ± 9%
	Whole Worms	25 ± 6%	21 ± 3%	14 ± 9%
HETE	Supernatants	58 ± 7%	89 ± 1%	90 ± 6%
	Whole Worms	42 ± 7%	11 ± 1%	10 ± 6%
Total	Supernatants	64 ± 7%	84 ± 2%	88 ± 7%
	Whole Worms	36 ± 7%	16 ± 2%	12 ± 7%

19. Eicosanoid Production in Adult Schistosomes.

Since we believe that host immuno-modulation via eicosanoid production in cercariae contributes to cercarial evasion of the host immune system, the same mechanism may be operative in adults. Thus, we finished a study evaluating secreted and non-secreted eicosanoid production in adult schistosomes and chemically transformed cercariae. Adult S. mansoni were recovered by perfusion of the hepato-portal system. ICR mice were sacrificed using pentobarbital and perfused with ice cold RPMI (Gibco) media (pH 7.2) containing 1% sodium citrate to prevent clotting. After collection, adults were washed in ice cold RPMI without heparin (pH 7.2) and placed in standard petri dishes to allow separation of male and female worms. Five males or five females were placed in 5ml polypropylene test tubes containing 2ml fresh RPMI supplemented

with 3mM linoleate (1uCi [^{14}C]-U-linoleate). Incubation was started within 2 hrs of perfusion. Cercariae (13,300) were incubated in 5 ml polypropylene test tubes containing 2 mls of Earles salts with lactalbumin hydrolysate (ELAC) supplemented with 3mM linoleate (1uCi [^{14}C]-U-linoleate). Cercariae were used within 2 hrs of shedding. All incubations were carried out at pH 7.2 for 1 hr at 37C in a shaking water bath. After 1 hr incubation tubes were centrifuged, the supernatant was removed and 8 mls of 100% methanol was added. The worms contained in the pellet were covered with 10 mls of 80% methanol and homogenized. Methanol served to stop eicosanoid biosynthesis and to start the extraction procedure. Extracts were overlaid with nitrogen and kept at -70C until HPLC or RIA analysis.

The results of HPLC analysis are given in Table XI and XII. As can be seen, adult males and females secrete most of the eicosanoids they produce, while cercariae secrete ~64%.

Table XII A Comparison of Eicosanoid Production (Secreted plus Non-Secreted) as Determined by RIA in Schistosoma mansoni

Eicosanoid	Cercariae	Adults Males	Adults Females
Species	(μg/mg soluble protein/hr)		
PGE*	0.043 ± 0.004	14.4 ± 4.44	137.0 ± 49.2
LTB_4	0.038 ± 0.004	17.3 ± 1.49	87.0 ± 15.7
5-HETE	0.086 ± 0.019	144.4 ± 23.2	157.2 ± 49.4

*This antibody cross-reacts with both PGE_1 and PGE_2.

The data reported here demonstrate that adult schistosomes secrete immunosuppressive eicosanoids of both the cyclo-oxygenase and lipoxygenase pathways. PGE has been detected by both HPLC and RIA. The HPLC data also show that the immunosuppressant 15-HETE is present in amounts exceeding that of the immunostimulant 5-HETE. In addition, other eicosanoids having potentially positive or negative effects on the immune system are also present. The regulation and physiological effects of these secreted eicosanoids are clearly an area for future study. While we do not believe at this time that eicosanoid production is the major mechanism responsible for keeping the adults and larval stages of the parasite "hidden" from the host immune system, we do believe that the evidence presented here suggests a supportive role for these products. Indeed, the intrinsic defense mechanism in the early schistosomule mentioned by a number of authors, may be eicosanoid-related or eicosanoid in fact. These data further strengthen our hypothesis that eicosanoids play a key role in cercarial invasion and maturation in the host.

CONCLUSIONS

In conclusion, we believe we have completed the scope of this contract with admirable results. We carried out intensive investigations into the biochemistry of cercarial penetration as a basis for which to develop a rational approach to chemoprophylaxis. Based on these studies we formulated an eicosanoid hypothesis, which became the basis for investigating known eicosanoid inhibitors for their prophylactic activity against the invasive form of Schistosoma mansoni. These data lead us to screen over 42 known eicosanoid inhibitors using an in vitro system which we developed. Of those compounds, we examined esculetin, praziquantel and ketoconazole for prophylactic effects in vivo using a mouse model. Of these three compounds, praziquantel showed prophylactic activity, but not of a long lasting effect. However, one of its lipid vehicles, Cremophor El, had lasting prophylactic activities. This lipid proved to be a strong eicosanoid inhibitor. This lead us to evaluate derivatives of Cremophor El for their ability to inhibit eicosanoid production and cercarial stimulation by linoleate. The prophylactic activity of four of these derivatives were investigated, of which Castor Oil proved superior.

The data presented here indicate that Castor Oil is a very effective prophylactic agent; however, its clinical applications are doubtful. This compound, does however give us a structure-activity basis from which to search for more promising compounds. In addition, it clearly indicates that chemoprophylaxis to schistosomiasis is possible.

LIST OF PUBLICATIONS RESULTING FROM DAMD 17-85-C-5180.

Articles Published

1. Fusco, A.C., B. Salafsky, K. Whitely and S. Yohe. 1987. Schistosoma mansoni: pH dependence of cercarial eicosanoid production, penetration and transformation. Exp. Parasitol. 64:139-146.

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8. Salafsky, B., A.C. Fusco, J. Mueller. In preparation. Schistosoma mansoni: The effect of Castor Oil and its derivatives as a chemoprophylactic agent. Exp. Parasitol.

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1. Fusco, A.C., B. Salafsky, K.D. Whitley and S. Yohe. Cercarial (Schistosoma mansoni) eicosanoid production, penetration, and transformation as a function of pH. ICOPA VI Brisbane, Australia, Aug. 1986.

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3. Fusco, A.C., B. Salafsky and K. Whitley. 1986. Schistosoma mansoni: Mouse skin eicosanoid production as a correlate of cercarial penetration. 61st Meeting of the American Society of Parasitologists, Dec. 1986.

4. Salafsky, B. and A.C. Fusco. The Effect of Praziquantel (PZQ) on Schistosoma mansoni Cercarial Pentration. ASPET, Honolulu, August 1987.

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8. Salafsky, B., A.C. Fusco, and L.H. Li. Praziquantel (PZQ) as a prophylactic agent aganist Schistosoma mansoni cercarial skin penetration. 36th Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles. Dec. 1987.

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GLOSSARY

Cercaria - The infective stage of Schistosoma mansoni. This stage is released from the snail intermediate host and is the stage that infects the human host via skin penetration processes.
EFAs - essential fatty acid, precursors to eicosanoid synthesis.
Eicosanoid- A generic term referring to both cyclo-oxygenase and lipoxygenase metabolites of arachidonic acid metabolism.
ELAC - Earles Salts with Lactalbumin hydrolysate.
EM - Electron microscopy
HETE - Hydroeicosatetraenoic acid.
HPLC - High Performance Liquid Chromatography.
KETO - ketoconazole.
LT - Leukotriene.
LTB_4 - Leukotriene B_4.
NEFAs - nonessential fatty acids
PG - Prostaglandin.
PGE - Prostaglandin E.
PZQ - praziquantel, the DOC for adult S. mansoni infections.
RIA - Radioimmunoassay.
Schistosoma mansoni - (Trematoda) A human parasite infecting the mesenteric veins of its host. This parasite has a complex two host life cycle. Asexual reproduction occurs in an infected snail while the sexual (adult) stages occur in the human host. The infective stage for the definitive host (man) is the cercarial stage.
Schistosomule - A stage in the life cycle of Schistosoma mansoni. This stage occurs after the cercaria penetrates the host skin and undergoes biochemical and morphological processes called transformation.
TLC - Thin Layer Chromatography.

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